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JOURNAL OF AGRICULTURAL RESEARCH

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ERRATA AND AUTHORS' EMENDATIONS

Page 43, lines 27 and 28, hyphens should be inserted after "Small" in both cases and after "smooth."
Page 43, fourteenth line from bottom, "(dO)" should be "(d)."
Page 45, legend for Figure 3, "large" in both cases should be "Large."
Page 258, seventh line from bottom, "×" after 2.269 should be "*X*."
Page 261, paragraph 3, line 2, omit "were grouped together, and plots."
Page 265, Table 7, last column, fraction line (shilling designation) should be omitted after "Diff." in the heading.
Page 267, fifth line from bottom, the first "+0.635" should be "+0.373."
Page 358, Fig. 2, "Terminal" should be omitted from legends for I and J.
Page 516, line 32, "Mellon's" should be "Millon's."
Page 517, line 30, "28°" should be "23°."
Page 608, Table 2, "Trioxymethylone" should be "Trioxymethylene."
Page 625, line 17, "nelanosae" should be "melanose."
Page 648, line 7, sentence should be "The fungus which was isolated from Chinese cabbage and from rutabaga infected cabbage."
Page 663, line 5, "Elliot" should be "Elliott." Throughout the article "*fumbriatum*" should be "*fumbriata*"
Page 741, third line from bottom; page 742, lines 10 and 16: "(0.75 mol.)," "(0.8 mol.)," "(0.9 mol.)," respectively, apply only to the divalent precipitants and should be doubled for the monovalent.
Page 742, line 1, Reference (19) is: Vogel, A., and Reischauer, C. 1859. In Jahresber. Pharm., v. 11, p. 3.
Page 770, line 5, "*Aphanobacter*" should be "*Aplanobacter*."
Page 782, line 7, "length" should be "index."
Page 943, line 4 below tabulation, add "em. Wr." after "Sacc."
Page 947, seventh line from bottom, "inconidiis" should be "in conidiis."
Page 948, second line from bottom, add "I" after "forma."
Page 993, in title of author, "Assistant" should be "Associate."
Page 1028, heading "Infected Soil" should be "Infested Soil."
Page 1075, Table 1, Lot 2, for sheep No. 393–961, under "Tensile Strength," "2,904" should be "2,094."
Table 2, first part, for the average value of "Average elongation," ".003" should be ".0038."
Table 2, second part, for sheep No. 541, under "Young's modulus," "9,54" should be "9,540."
Page 1085, Table 5, Lot 2, for sheep No. 492, under "Tensile Strength," "176,190" should be "176,100."
Page 1089, Literature Cited No. (3), "life weight" should be "live weight."
Page 1091, in title, "W. & C." should be "Schlecht."
Page 1146, line 29 and Literature Cited No. (27), "Jochinai" should be "Tochinai."
Page 1148, in last column, "20 hours ^{b}" should be "20 hours ^{c}."
Page 1150, line 5, add "(see fig. 1)" after "flagging" to replace same expression in line 3 of last paragraph.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 33 WASHINGTON, D. C., JULY 1, 1926 No. 1

EXPERIMENTS WITH CERTAIN ARSENATES AS SOIL INSECTICIDES[1]

By B. R. LEACH

Associate Entomologist, Japanese Beetle Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

Three years ago a casual experiment, conducted in connection with certain other investigations of insecticides for the treatment of soil, demonstrated the fact that the larva of *Popillia japonica* Newm. dies when it feeds in soil containing a sufficient amount of certain arsenates. The constant ingestion of soil by the larva in the course of its feeding is conducive to rapid toxic action under the conditions referred to. The result of this experiment was of interest in that it offered a possible answer to certain problems in connection with the prevention of spread of the Japanese beetle.[2] Subsequent experimental work was therefore carried out at the Japanese Beetle Laboratory, Riverton, N. J., for the purpose of ascertaining the effect of various arsenates upon the soil, plant, and insect.[3]

THE ARSENATES EMPLOYED

Acid lead arsenate ($PbHAsO_4$)

	Per cent
Total arsenic oxide (As_2O_5)	31. 97
Lead oxide (PbO)	63. 72
Water of constitution and undetermined	4. 31
Total	100. 00
Soluble arsenic oxide (As_2O_5)	[4] . 05

Basic lead arsenate ($Pb_4(PbOH)(AsO_4)3H_2O$)

Total lead oxide (PbO)	74. 22
Total arsenic oxide (As_2O_5)	23. 54
Water-soluble arsenic oxide (As_2O_5)	. 03
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	. 02

[1] Received for publication Dec. 4, 1925; issued July, 1926.

[2] The Japanese beetle spends the major portion of its annual life cycle as a larva or grub in the soil, transforming to the beetle stage in midsummer and depositing eggs in the soil for the larvae of the succeeding generation.

[3] The idea of applying arsenic to soil to kill insects present apparently was first conceived in the course of experiments by the Cornwall (England) County Council in 1899. A brief account of those experiments is given in the following report: HAWK, W. WIREWORMS. Cornwall County Council, Ann. Rpt. Agr. Expts. (1898) 3: 45–48. 1899. More recently, experiments with white arsenic (arsenious acid) have been made in Queensland, Australia, in connection with the control of grubs attacking the roots of sugar cane. See JARVIS, E. ON THE VALUE OF POISON BAIT FOR CONTROLLING CANE GRUBS. Queensland Bur. Sugar Expt. Stas. Div. Ent. Bul. 4, 14 p., illus. 1916.

[4] Analyses made by Insecticide and Fungicide Board, Bureau of Chemistry, United States Department of Agriculture.

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Magnesium arsenate

	Per cent
Total arsenic oxide (As_2O_5)	44. 33
Total arsenic oxide equivalent to $Mg_3(AsO_4)$	67. 65
Water-soluble arsenic oxide ($(AsO_4)_2$)	1. 03
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	. 67
Total arsenic oxide equivalent to magnesium arsenate	67. 65
Magnesium oxide in excess of arsenic oxide (MgO)	8. 43
Calcium oxide (CaO)	1. 98
Oxides of iron and aluminum (R_2O_3)	1. 20
Acid-insoluble ash (silicious material)	. 68
Water of hydration (CO_2), organic matter, etc. (by diff.)	20. 06

Sample is a commercial grade of magnesium arsenate containing a small amount of casein.

Zinc arsenate ($Zn_3(AsO_4)_2$)

	Per cent
Total zinc oxide (ZnO)	43. 26
Total arsenic oxide (As_2O_5)	38. 35
Total arsenic oxide equivalent to zinc arsenate ($Zn_3(AsO_4)_2$)	79. 07
Water-soluble arsenic as arsenic oxide (As_2O_5)	. 60
Water-soluble arsenic as arsenic oxide equivalent to metallic arsenic (As)	. 39

Hypothetical combinations

Zinc arsenate ($Zn_3(AsO_4)_2$)	79. 07
Zinc oxide (ZnO) in excess of arsenic oxide	2. 54
Loss on ignition	16. 58
	98. 19

Copper arsenate ($Cu_3(AsO_4)_2$)

Arsenic oxide (As_2O_5)	46. 47
Copper oxide (CuO)	37. 92
Sodium oxide (Na_2O)	4. 30
Iron oxide (Fe_2O_3)	. 22
Loss on ignition	9. 74
Water-soluble arsenic as arsenic oxide (As_2O_5)	3. 10
Water-soluble arsenic as arsenic oxide equivalent to metallic arsenic (As)	2. 02

Hypothetical combinations

Sodium arsenate (Na_3AsO_4)	9. 61
Tri-copper arsenate ($Cu_3(AsO_4)_2$)	55. 54
Di-copper arsenate ($CuHAsO_4$)	24. 63
Water of constitution, etc. (diff.)	10. 22
	100. 00

Sample is essentially tri-copper arsenate, di-copper arsenate, sodium arsenate, and water of constitution.

Calcium arsenate ($Ca_3(AsO_4)_2$)

	Per cent
Total arsenic oxide (As_2O_5)	40. 83
Total arsenic oxide equivalent to tri-calcium arsenate ($Ca_3(AsO_4)_2$)	70. 70
Water-soluble arsenic oxide (As_2O_5)	3. 63
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	2. 37

Ferric arsenate ($FeAsO_4$)

Ferric oxide (Fe_2O_3)	33. 22
Total arsenic oxide (As_2O_5)	40. 56
Total arsenic oxide equivalent to ferric arsenate ($FeAsO_4$)	68. 73
Water-soluble arsenic oxide (As_2O_5)	. 17
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	. 11

No ferrous iron detected.

Hypothetical combinations	Per cent
Ferric arsenate ($FeAsO_4$)	68. 73
Ferric oxide (Fe_2O_3) in excess to combine with As_2O_5	5. 04
Loss on ignition (water of constitution and moisture)	25. 36
	99. 13

TOXICITY OF ARSENATES TO LARVA IN THE SOIL

The various arsenates mentioned above were tested to determine their toxicity to the larva of the Japanese beetle. This work was done during the late winter under greenhouse conditions, the soil temperature averaging about 60° F. In each case the specific quantity of the arsenate was thoroughly mixed with the required amount of soil, and the mixture was placed in 4-inch flowerpots on the greenhouse bench, each test embracing 5 pots. The pots of soil were then moistened, and 24 hours later they were infested with 4 larvae per pot. The results of these tests are given in Table 1.

TABLE 1.—*Toxicity to third instar larvae of the Japanese beetle of various arsenates when mixed with soil (soil temperature,[a] 60° F.)*

Compound	Amount per 4-inch pot	Equivalent amount per acre	Days required to kill all larvae
	Gms.	*Lbs.*	
Acid lead arsenate	0. 275	500	40
Do	. 55	1, 000	18
Do	. 825	1, 500	10
Do	1. 10	2, 000	7
Do	1. 65	3, 000	5
Basic lead arsenate	0. 55–2. 75	1, 000–5, 000	[b]
Zinc arsenate	. 55–2. 75	1, 000–5, 000	[c]
Calcium arsenate	. 55–2. 75	1, 000–5, 000	[d]
Copper arsenate	. 55–2. 75	1, 000–5, 000	[c]
Magnesium arsenate	. 55–2. 75	1, 000–5, 000	[b]
Ferric arsenate	. 55–2. 75	1, 000–5, 000	[b]

[a] The speed of kill for a definite concentration of arsenate in soil depends upon the soil temperature; the larva is relatively inactive below 50° F.
[b] Larvae not killed.
[c] Killed somewhat more slowly than acid lead arsenate.
[d] About the same as acid lead arsenate.
[e] Same as zinc arsenate.

The basic lead arsenate, the magnesium arsenate, and the ferric arsenate were not toxic to the larvae. The zinc arsenate and the copper arsenate were slower in killing the larvae than acid lead arsenate and calcium arsenate. Summarizing the results, the experiments indicate, from the standpoint of larval mortality, that acid lead arsenate is the best arsenate for killing larvae in the soil.

OUTDOOR EXPERIMENTS WITH ACID LEAD ARSENATE

EXPERIMENT NO. 1

On April 21, 1922, four wooden frames, 21 inches wide, 36 inches long, and 8 inches deep, with a 16-mesh wire-screen bottom, were sunk in the soil outdoors, the top of the frame being level with the surface of the soil. Ordinary potting soil was then placed in each frame to a depth of 4 inches and infested with 100 third-instar larvae of the Japanese beetle per frame. Frames Nos. 1, 2, and 3 were then

filled with soil containing acid lead arsenate at the rate of 1,000, 2,000, and 3,000 pounds per acre, respectively. Frame No. 4 was filled with ordinary soil containing no arsenate of lead.

At this time (April 21) the soil temperature was low (40° F.), and the larvae were inactive, but by May 2 the soil had warmed up considerably and the larvae were moving into the upper 4 inches of arsenated soil. The examinations of the soil in the various frames made at subsequent intervals are given in Table 2.

TABLE 2.—*Mortality of third-instar Japanese beetle larvae in outdoor soil arsenated to a depth of 4 inches, 100 larvae being used in each test (experiment begun April 21, 1922)*

Date of examination	1,000 pounds		2,000 pounds		3,000 pounds		Control	
	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches
May 8	60	20	32	28	24	12	48	52
May 15	8	24	8	24	0	36	40	56
May 22	4	16	6	4	4	6	46	46
May 31	4	10	2	2	2	4	41	43
June 12	0	0	0	0	0	0	[a] 29	[b] 17

[a] Eleven others had pupated. [b] Twenty-four others had pupated.

In the untreated soil the larvae remained normal and were pupating by June 12, while in the arsenated soil the larvae were succumbing to the poison and by June 12 all were dead.

EXPERIMENT NO. 2

Wire-screen cages, 16-mesh wire, 5 feet square by 5 feet in height, were employed. These were set up in a row outdoors on July 1, 1922, with the cage baseboards sunk in the ground. The soil in the individual cages was treated on July 2, 1922, as shown in Table 3.

TABLE 3.—*Results of experiment No. 2 with larvae of the Japanese beetle in arsenated soil*

Cage No.	Treatment of soil in cage made July 2, 1922	Number of larvae found Sept. 1	
		In untreated soil of cage	In treated soil of cage
1	Control, soil not treated	475	
2	Soil received acid lead arsenate [a] at rate of 1,000 pounds per acre		0
3	Soil received acid lead arsenate at rate of 2,000 pounds per acre		0
4	One-half soil area of cage received acid lead arsenate at rate of 1,000 pounds per acre; remainder of soil area untreated [b]	268	0
5	Same as 4, but arsenate employed at rate of 2,000 pounds per acre	231	0

[a] Spread evenly over surface of the soil and worked in to a depth of 4 inches with rake and hoe.
[b] Treated and untreated portions separated by a board sunk into the soil.

Twelve hundred and fifty Japanese beetles were placed in each cage, in lots of 250 at intervals of 4 days, beginning July 5, and smartweed was supplied as food. The beetles deposited eggs indiscriminately in both the treated and the untreated soil, but the larvae coming from those deposited in the treated soil died very shortly after hatching.

EFFECT OF ARSENATED SOIL ON PLANTS

The effect of arsenated soil on plants depends upon the nature of the arsenate. The experiments at the Japanese Beetle Laboratory indicate that the arsenates nontoxic to the larvae are nontoxic to plants. The basic arsenate of lead, the magnesium arsenate, and the ferric arsenate are typical examples in this respect. When mixed with soil at the rate of 3,000 pounds per acre, these arsenates did not seem to inhibit the growth of the common garden bean to the slightest extent.

The arsenates which proved toxic to the larvae also proved toxic to plants, although there were some outstanding exceptions. The arsenates of copper, zinc, and calcium were decidedly toxic to plants, only one plant (*Pteris wimsetti*) withstanding them to any appreciable extent. It was, however, with acid arsenate of lead that the outstanding instances of plant resistance to a larva-killing arsenate were encountered. The presence in the soil of relatively large amounts of this compound is tolerated to a surprising extent by nasturtium, snapdragon, *Pteris wimsetti*, *Vinca minor*, *Clematis paniculata*, and certain of the grasses. These plants were equal in growth to the controls, and in several instances were superior to them, notably in the case of *Pteris wimsetti*. Nasturtium and snapdragon bloomed normally when grown in soil containing acid lead arsenate at the rate of 2,000 pounds per acre. On the other hand, certain plants such as *Salvia splendens*, Calendula, and strawberry made no growth in soil so treated, while there are numerous plants which have some resistance to this arsenate, the degree of resistance varying with the species. In general, the results of the experiments indicate that the plant phase of the problem offers much of fundamental interest to the plant physiologist.

SOIL ACTION UPON ACID LEAD ARSENATE [5]

Acid lead arsenate undergoes chemical change when mixed with moist soil, the nature of the change being indicated by the reaction of the plant and insect. In all probability the compound is acted upon by the soil solution, with the consequent formation of insoluble basic arsenates and soluble arsenates, with the latter ultimately leaching away or being absorbed or possibly rendered impotent by buffer reactions. The presence of soluble arsenic is indicated by the stunting of plant growth, while the formation of insoluble basic arsenates is indicated by a certain degree of loss of toxicity toward the larvae.

[5] For the most complete account of the action of the soil solution upon acid arsenate of lead see the following: STEWART, J. SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 1. Soil Sci. 14: 111-118. 1922.
——— and SMITH, E. S. SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 2. Soil Sci. 14: 119-126, illus. 1922.

This decrease of toxicity toward the larvae, however, depends upon the amount of acid lead arsenate applied to the soil, as indicated in Table 4.

TABLE 4.—*The decrease in toxicity to the larvae of the Japanese beetle caused by the action of the soil solution upon the arsenate of lead applied*

Pounds per acre	Number of days required to kill larvae introduced into soil which had been mixed with the arsenate for various periods of time			
	Immediately after mixing	After being mixed 1 year	After being mixed 2 years	After being mixed 3 years
500	40	(a)	(a)	(a)
1,000	18	40	40	40
1,500	10	(a)	(a)	(a)
2,000	7	10	10	10

a No data.

It will be noted that there is a certain loss in toxicity to the larvae due to the breakdown of a part of the arsenate in the soil, whereupon the degree of toxicity persisting tends to remain constant. In practice, therefore, it would seem advisable either to apply a sufficient initial quantity of arsenate to allow for the loss in toxicity of a portion, or to reinforce a small initial application with a subsequent supplementary one. How long the toxicity to the larvae of the larger applications will endure, and the various factors influencing this are not known, but experiments are now under way to obtain data along these lines.

THE PRACTICAL USE OF ACID LEAD ARSENATE AS A SOIL INSECTICIDE [6]

The experimental data outlined above have been applied to the solution of certain problems, and a considerable quantity of acid lead arsenate is now being used in the quarantined area. At present acid arsenate is being used extensively for freeing soil of larval infestation and maintaining it in that condition.

Large quantities of field-grown perennials are dug in the early fall by certain of the local nurseries after the beetle flight is over, potted in clean soil, and the pots plunged into cold frames the soil of which has been treated one month previously with acid lead arsenate at the rate of 1,500 pounds per acre [7] (3.5 pounds per square foot). The arsenate is spread evenly on the surface and worked in to a depth of 4 inches, the work being preferably done when the soil is on the dry side. Under the system just described this potted stock can be held for future shipment with no danger of infestation. The pots could

[6] The foregoing account of this work was written in August, 1924. Since that time the writer, assisted by J. W. Lipp, of the Japanese Beetle Laboratory, has continued the work, paying especial attention to the use of acid lead arsenate as a poison for the larvae of the Japanese beetle in lawns and golf greens, which work has shown that many of the finer grasses, notably the bents, do remarkably well in poisoned soil while the grub is effectively controlled. Extensive grass plot tests are now under way along these lines. See LEACH, B. R., and LIPP, J. W. A METHOD OF GRUB-PROOFING TURF. Bul. Green Sec. U. S. Golf Assn. 6:34-39, illus. 1926.

[7] The narrow paths between the cold frames are also treated.

not be placed in untreated soil without the ever-present danger of larvae working their way into the pots through the holes at the bottom.

Several of the local nurseries have also treated areas of ground of an acre or less with arsenate of lead in order to have available a piece of ground free of larvae for the purpose of heeling in deciduous shrubbery, iris, peonies, funkia, etc., during the shipping season. In treating larger areas of this sort the arsenate is applied to the plowed ground (1,500 pounds per acre) with a fertilizer drill and thoroughly worked over with disk and spiketooth harrows, the work being done when the soil is on the dry side and a month before the ground is to be used for the above purpose. Soil taken at random from areas so treated and placed in a flowerpot, within two weeks, gave 100 per cent mortality of larvae placed therein. Moreover, no larvae were found in the soil so treated 14 months later at the conclusion of the next year's beetle flight, indicating that the larvae emanating from eggs deposited therein quickly succumbed. Soil treatment of this sort is supervised by the quarantine officials, and thoroughness of application is insisted upon.

The ultimate effect of lead arsenate on soil so treated is not known at present, and it is urged that only the minimum area of ground necessary for the conduct of business be so treated.

EXPERIMENTS WITH COATED ARSENATE OF LEAD

In view of the chemical change which acid lead arsenate undergoes in the soil and the complications arising thereby in the use of this compound as a soil insecticide, the idea was conceived of coating the impalpable particles of the arsenate with materials which would prevent the chemical action of the soil solution upon the arsenate but which would be broken down by the gastric juices in the intestinal tract of the larvae, thereby exposing the arsenate of lead and permitting its toxic action.

Oils, fats, waxes, and various plant, animal, and mineral compounds of the type suitable for this purpose have been tested in this connection during the last three years, a method of coating suggested by P. A. van der Meulen of Rutgers College being employed. This method consists essentially in dissolving the coating material in some suitable solvent such as benzene or alcohol, stirring in the arsenate of lead, and evaporating to dryness. Perfect coatings can be obtained by this method, 100 grams of lead arsenate being coated with 5 to 15 grams of coating material. With a larger proportion of the coating material the final product is likely to be too sticky for use in soil.

The results of the experimental work with coated arsenate of lead to date have been negative; the coating materials have been destroyed by the soil action, thereby exposing the arsenate of lead to the soil action. Until recently it was thought that the destruction of the coat was entirely a chemical action, but it is rather far-fetched to attribute the loss of such a chemically inert coating material as paraffin to the action of the relatively dilute soil solution. In this connection tests made at this laboratory by W. E. Fleming indicate that certain soil organisms are responsible for the loss of the paraffin coating. It is therefore proposed to continue the work on the coating of arsenate of lead for use in soil, special attention being given to both the chemical and soil-biological aspects of the problem.

SUMMARY

The larva of the Japanese beetle, *Popillia japonica* Newm., dies when it feeds in soil containing a sufficient amount of certain arsenates. The compounds tested in this connection were basic and acid lead, magnesium, zinc, copper, calcium, and ferric arsenates. The basic lead, magnesium, and ferric arsenates were nontoxic to the larva. Acid lead arsenate proved the most satisfactory from the standpoint of larval toxicity. At a soil temperature of 60° F. the larva succumbs to the acid lead arsenate in a certain number of days when feeding in soil treated with the compound, the number of days required to kill the larva depending upon the concentration of arsenate. The arsenates nontoxic to the larva proved also to be nontoxic to plants. Of the arsenates lethal to the larva acid lead arsenate was least toxic to plants. Nasturtium, snapdragon, *Pteris wimsetti*, *Vinca minor*, *Clematis paniculata*, and *Poa annua*, grew normally in acid lead arsenate applied at the rate of 2,000 pounds per acre. Many plants showed considerable tolerance for the compound, while many others tested were severely checked in growth. Acid lead arsenate undergoes chemical change when mixed with moist soil, the tendency being toward the formation of basic arsenates. Acid lead arsenate applied at the rate of 1,500 pounds per acre is now being used to a considerable extent by nurseries in the area infested by the Japanese beetle as a means of freeing soil of the larva and keeping it free. The results of experiments in coating the particles of lead arsenate for the purpose of resisting soil action have been negative so far.

WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII IN 1921[1]

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INTRODUCTION

The Mediterranean fruit fly (*Ceratitis capitata* Weidemann), considered by many to be the most destructive insect pest of tropical and subtropical fruits, was first found in the Hawaiian Islands in 1910. Since then various methods of bringing it under control have been tried. Clean culture (the destruction of all host fruits), spraying with poisoned baits, trapping, and covering fruits before infestation occurs, have all been thoroughly tested. No practicable method of control, for general Hawaiian conditions, has yet been discovered. However, under the peculiar conditions existing in the Kona coffee fields on the island of Hawaii, introduced parasites have so decreased the number of fruit flies that the coffee cherries are now nearly free from infestation.

In Hawaii, natural enemies accomplish a greater degree of control than any other agency. In a previous publication (*7*)[2] it was shown that the ant *Pheidole megacephala* Fab. may destroy from one-third to four-fifths of the fruit-fly larvae developing in fruits which have fallen to the ground. Other natural enemies of minor importance occur; but the most effective natural control has been accomplished by four species of larval parasites which were introduced by the Territory of Hawaii. Two opiines, *Opius humilis* Silvestri from Africa and *Diachasma tryoni* Cameron from Australia, were introduced in 1913; and one opiine, *Diachasma fullawayi* Silvestri, and one chalcid, *Tertastichus giffardianus* Silvestri, from Africa in 1914.

The Bureau of Entomology of the United States Department of Agriculture has kept careful records of the degree of parasitism attributable to each of the four species since their liberation in 1914. Records of the amount of infestation of different fruits by the fruit fly since 1916 have also been kept. These records have been made from almost daily collections of fruits from the vicinity of Honolulu, from which careful counts of parasitized and nonparasitized flies have been made. Such records are a good guide to the progress of each parasite under Hawaiian conditions, and give definite information on the number of flies destroyed by parasites each year.

RECORDS OF PARASITISM

The results of these observations for 1914 to 1920, inclusive, have been published (*1*, *2*, *4*, *6*, *8*, *9*) annually, except those for 1919 and 1920, which were published in one paper.

[1] Received for publication Dec. 12, 1925; issued July, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 15.

The data in Table 5 show that since 1916 the yearly variation in parasitism has not been great, but that considerable variation occurs over a period of three years. The parasitism during 1916 was 33.2 per cent, increasing to 55.8 per cent in 1918; in 1919 it dropped to 38.2, and increased in 1921 to 56.4. Abundance of adult flies in the field is indicated by the numbers of maggots in the host fruits. In 1913 and 1914, before parasites had become established, *Ceratitis capitata* evidently reached its maximum abundance. No statistical records which might give the exact degree of infestation of different host fruits were compiled before the liberation of the parasites. However, numerous statements made to the writers by fruit growers, entomologists, and others whose observations were considered reliable, all definitely agree that maximum infestation occurred at that time, and also that there was a very noticeable decrease in infestation during the first three years after the liberation of parasites.

If records of the average number of maggots per fruit are made from a sufficiently large number of fruits each year, they will show the comparative abundance of adults in the field from year to year. Records of this kind were begun in Honolulu in 1916. Table 1 gives the average infestation of each fruit in 1921, together with the number of fruits and larvae under observation. During the year 133,646 fruits were collected, from which 677,575 larvae were taken, this collection being typical of the large numbers of fruits from which the records have been made each year.

TABLE 1.—*Extent of infestation of host fruits by larvae of Ceratitis capitata in Hawaii in 1921*

Host fruit	Number of fruits collected	Number of larvae emerging	Average number of larvae per fruit
Indian almond (Terminalia catappa)	30, 968	325, 865	10. 5
Mango (Mangifera indica)	1, 525	5, 961	3. 9
Coffee (Coffea arabica)	4, 173	1, 693	.4
Strawberry guava (Psidium cattleianum)	3, 575	12, 176	3. 4
Black myrobalan (Terminalia chebula)	12, 924	91, 048	7. 0
French cherry (Eugenia uniflora)	16, 901	31, 546	1. 9
West Indian medlar (Mimusops elengi)	4, 058	26, 737	6. 6
Sapodilla (Achras sapota)	249	1, 985	8. 0
Carambola (Averrhoa carambola)	222	222	1. 0
Loquat (Eriobotrya japonica)	701	3, 168	4. 5
Chinese orange (Citrus japonica)	49, 118	127, 947	2. 6
Guava (Psidium guajava)	6, 306	42, 052	6. 7
Orange (Citrus aurantium)	1, 467	3, 240	2. 2
Tangerine orange (Citrus nobilis)	1, 065	3, 290	3. 1
Noronhia emarginata	201	365	1. 8
Ochrosia elliptica	193	280	1. 5

The data in Tables 1 and 2 were obtained from host fruits gathered for the purpose of obtaining records of fruit-fly parasitism, and only ripe, well-infested fruits were collected. Edible host fruits picked for market are removed from the trees in the earliest stages of maturity, when they have been exposed to a minimum of fruit-fly attack and show very little, in some cases no, infestation. Tables 1 and 2, therefore, are not records of the infestation of fruits intended for commercial purposes.

Table 2, for purposes of comparison, gives the average number of maggots per fruit for each year from 1916 to 1921, inclusive, together with the average per fruit for the 6-year period. The extent of these observations is indicated by the fact that 788,826 fruits

were collected, from which 2,830,310 larvae were taken. A comparison of the average infestation per fruit in all species of hosts during each year, with the average infestation for the 6-year period, shows that the proportion of hosts with more than average infestation steadily decreased from 1916 to 1920, and greatly increased in 1921. In 1916, 47.1 per cent of the total number of species of host fruits had a higher average number of larvae per fruit than the average for the 6-year period shown in the last column of Table 2. Similar comparisons for the five years which followed show that in 1917 40 per cent of the host fruits were over the 6-year average in infestation; in 1918, 35.3 per cent; in 1919, 33.3 per cent; in 1920, 31.6 per cent; and that in 1921 the number increased to 68.8 per cent. The unusually high infestation of fruits during 1921 was accompanied by the greatest abundance of parasites since their liberation, resulting in a total parasitism for the year of 56.4 per cent.

TABLE 2.—*Average number of Ceratitis capitata larvae per fruit in Honolulu for the period 1916 to 1921, inclusive, and for each year of the period*

Host fruit	Average number of larvae per fruit						
	1916	1917	1918	1919	1920	1921	For the 6-year period
Indian almond	9.5	8.0	9.9	8.4	5.5	10.5	8.5
Mango	1.7	8.1	24.4	3.7	3.5	3.9	4.0
Coffee	.5	.8	.6	.4	.6	.4	.6
Strawberry guava	1.6	2.0	1.3	1.2	1.2	3.4	1.4
Black myrobalan	7.0	5.9	4.8	4.7	6.9	7.0	6.1
French cherry	.8	1.0	1.0	.9	1.4	1.9	1.2
Satin leaf (Chrysophyllum olivaeforme)	2.0	3.4	3.2	5.0	4.7		3.3
Rose apple (Eugenia jambos)	5.5	8.8	6.6		11.5		9.5
West Indian medlar	5.3	1.8	2.5		.4	6.6	3.7
Sapodilla		4.7				8.0	7.0
Carambola	1.3	1.6	.9	.05	.2	1.0	.7
Yellow oleander (Thevetia neriifolia)	3.6	5.7	6.0	1.7	1.9		3.6
Loquat		2.6	1.8	3.4		4.5	2.5
Chinese orange	3.1	1.8	1.8	2.5	2.4	2.6	2.4
Guava	6.8	4.5	8.5	9.8	7.2	6.7	7.8
Orange				3.3	6.0	2.2	3.3
Tangerine orange					.9	3.1	2.1
Lime (Citrus medica limetta)					1.2		1.2
Noronhia emarginata	1.7		1.1		.1	1.8	1.6
Orchrosia elliptica	3.1	23.5				1.5	5.6
Kamani (Calophyllum inophyllum)	3.3	2.4	2.4	6.0	4.9		3.6
Peach (Amygdalus persica)	20.5	15.2	22.4	13.6	15.6		18.4
White sapote (Casimiroa edulis)		7.6					7.6
Wampi (Clausena wampi)		.2					.2

The data in Table 3 are recorded to show the ability of each species of parasite to attack its host in different species of fruits, and to indicate any preference on the part of each parasite for particular host fruits. *Opius humilis*, *Diachasma tryoni*, and *D. fullawayi*, all of which oviposit in the larvae of *Ceratitis capitata* from the outside through the skin of the fruit, are capable of parasitizing only those maggots which are very close to the outer surface, where they are within reach of the parasite's ovipositor. Consequently, the ability of these opiines to attack their host is much greater in small fruits and in those containing a large seed covered by a thin layer of pulp into which the maggots can not penetrate deeply. The ability of *Tetrastichus giffardianus* to parasitize *C. capitata* depends more upon the nature of the fruit than does that of the opiines. This parasite enters the fruit and searches out the host larvae in which to oviposit, and a crack or other opening in the skin is necessary before it can reach its host.

TABLE 3.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1921*

Host fruit	Month	Number of larvae emerging during first 2 to 6 days	Percentage of parasitism				
			Opius humilis	Diachasma tryoni	Diachasma fullawayi	Tetrastichus giffardianus	Total
Indian almond	April	4,440	8.3	6.4	0.1	0.5	15.3
	May	15,676	19.4	58.7	1.3	1.8	81.2
	June	2,750	25.0	34.4	.8	6.6	66.8
	July	8,487	3.6	28.3	4.2	19.5	55.6
	August	12,976	5.9	21.9	4.2	39.7	71.7
	September	5,431	6.3	28.4	1.5	38.9	75.1
	October	3,904	3.1	31.9	.5	13.3	48.8
	November	4,253	3.4	26.3	1.2	20.4	51.3
	December	673	6.5	28.5	3.0	26.2	64.2
Mango	April	220	9.1	5.9	8.2	3.2	26.4
	May	22		9.1			9.1
	June	171	3.5	11.1	8.8	10.0	33.4
	July	325	2.2	22.2	9.2	6.5	40.1
Coffee	January	41	19.5	2.4	4.9		26.8
	February	400	12.3	7.7	19.7		39.7
Strawberry guava	do	122	.8			.8	1.6
	March	230	11.3	12.2	13.9	1.3	38.7
	April	471	3.4	4.2	3.6	.2	11.4
	June	392	2.5	21.7	27.8	25.5	77.5
	July	400	3.0	34.5	24.0	21.2	82.7
	October	197	6.7	19.3	1.0	46.2	73.2
Black myrobalan	February	1,878	.3	7.1	.5	15.3	23.2
	October	4,127	.7	10.4	7.7	.6	19.4
	November	2,051	1.6	15.3	12.9	4.8	34.6
French cherry	January	526	12.3	38.0	10.5	4.4	65.2
	February	1,199	5.5	36.5	28.2	12.9	83.1
	March	13	7.7		23.1	23.1	53.9
	June	262	3.8	44.7	31.7		80.2
	July	76	2.5	21.1	25.0	9.2	57.8
	September	811	1.2	19.8	36.7	3.6	61.3
	October	2,058	1.7	32.4	46.1	3.1	83.3
	November	1,483	4.6	19.2	17.8	3.5	45.1
West Indian medlar	March	330			.3		.3
	April	1,563	2.1		.7	.5	3.3
	May	168	5.4	6.4		1.2	13.0
Sapodilla	June	170	2.4	1.8	1.8	18.2	24.2
	July	44				40.9	40.9
Carambola	September	17		5.9		58.8	64.7
	October	73	6.9			64.4	71.3
Loquat	February	559	1.2	21.1	36.3	1.1	59.7
Chinese orange	January	69	1.5	10.1	4.4	7.2	23.2
	February	467	1.3	4.1	2.4	1.7	9.5
	March	636	5.8	1.4	4.4	3.3	14.9
	April	757	8.1	3.8	8.1	4.6	24.6
	May	994	5.8	17.3	3.9	1.9	28.9
	June	248	8.9	10.5	.8	20.1	40.3
	July	200	3.0	8.0	1.5	37.0	49.5
	August	33		6.1		18.2	24.3
	September	38	7.9	2.7		7.9	18.5
	October	15	13.3			13.3	26.6
	November	205	2.4	2.4	.5	8.3	13.6
	December	477	5.0	6.7	2.1	1.9	15.7
Guava	January	61		1.6		22.9	24.5
	February	245		12.7	9.0	6.5	28.2
	March	558	1.6	11.8	1.6	37.3	52.3
	April	349	5.7	8.3	2.6	42.7	59.3
	May	548	.4	14.2	5.5	37.0	57.1
	June	359	1.4	9.5	3.9	20.3	35.1
	July	138	8.0	10.9	5.1	31.1	55.1
	August	627	4.0	10.7	4.1	36.2	55.0
	September	112		24.1		4.5	28.6
	October	168		4.2	11.3	19.6	35.1
	November	1,168	2.3	1.0	1.1	68.8	73.2
	December	459	1.7	.4	2.0	65.8	69.9
Orange	January	97	2.1	5.1		7.2	14.4
	February	96	1.0	7.3		15.6	23.9
	March	46				37.0	37.0
	April	6					
	May	27		3.7	3.7	14.8	22.2
	June	34	2.9		2.9	41.2	47.0
	July	7			14.3		14.3
	September	39	2.6	7.7	5.1	43.6	59.0
	October	6				50.0	50.0
Mandarin orange	May	31	6.4	22.6	3.2	9.7	41.9
	June	63	3.2	20.6		1.6	25.4
	September	169	2.4	.6	1.2	12.4	16.6
Noronhia emarginata	July	75		1.3			1.3

Table 3 clearly shows this variation in effectiveness in different fruits. Indian almond (*Terminalia catappa*) is a good example of a fruit having a large seed and thin pulp. Its seed is from 1 to 2 inches long, about 1 inch wide, and ½ to ¾ inch thick, and is surrounded by ⅛ to $\frac{3}{16}$ inch of pulp, which is firm in texture and does not break easily when the fruit falls to the ground. It is usually well infested with maggots, and is available for collection about Honolulu nearly every month of the year. In this fruit, during 1921, the total parasitism by the opiine parasites was greater than that of *Tetrastichus giffardianus* for seven months and less for two months. Guava (*Psidium guajava*) and orange (*Citrus aurantium*) exemplify the large pulpy type of fruits in which *T. giffardianus* is more effective than the parasites which attack the maggots from the outside. When guavas ripen they fall off and often crack open upon impact with the ground, or there are holes in them made by birds or certain insects which allow *T. giffardianus* to enter freely and attack its host. Infested oranges, although they do not usually break open when they hit the ground, contain breathing holes made by fruit-fly larvae (*3*) through which *T. giffardianus* finds access to its host. In 1921 the parasitism of *Ceratitis capitata* by *T. giffardianus* in guavas was greater than the total parasitism by the three braconid parasites for 10 months and less for two months. In oranges, in 1921, it was greater for six months, the same for one month, and less for one month. The work of *T. giffardianus* is of special value because it attacks its host very readily in fruits where the maggots are inaccessible to the other three species, and particularly in guavas, which is the most abundant and widely distributed host of the fruit fly in Hawaii.

The percentages of parasitism shown in Table 3 indicate that all four parasites will attack their host in any fruit where the maggots are accessible, showing no great preference for maggots in one species of fruit over those in another. *Diachasma fullawayi*, however, has attacked the larvae in French cherry (*Eugenia uniflora*), strawberry guava (*Psidium cattleianum*), loquat (*Eriobotrya japonica*), and coffee (*Coffea arabica*), much more heavily than it has the larvae in other fruits.

Table 4, giving the percentages of parasitism by each species in all fruits under observation for each month in the year, shows the efficiency of each parasite during all seasons. In 1921 *Opius humilis* was the most effective species for one month, *Diachasma tryoni* for six months, and *Tetrastichus giffardianus* for five months. Similar tables previously published (*4*, *6*, *8*, *9*) show a steady increase in the relative importance of *T. giffardianus*. In 1915 and 1916 it was neither first nor second in amount of parasitism during any month. In 1917 and 1918 it was first in no month, but was second in relative importance during four months of each year. In 1919 it was first for one month and second for five months. In 1920, it was first for two months and second for one month, whereas in 1921 it had increased to the point where it was the most valuable parasite of the four for five months and the second most valuable for one month. Much of the parasitism by this species has been in the more fleshy fruits, where the opiine parasites work with difficulty, so that its great increase during 1921 considerably increased the total parasitism of *Ceratitis capitata* for that year.

TABLE 4.—*Total parasitism of Ceratitis capitata larvae from all fruits collected in Hawaii in 1921 (averaged by months)*

Month	Number of larvae	Percentage of parasitism				
		Opius humilis	Diachasma tryoni	Diachasma fullawayi	Tetrastichus giffardianus	Total
January	794	9.6	26.9	7.6	6.2	50.3
February	4,966	2.7	15.7	13.3	9.8	41.5
March	1,813	4.0	5.7	4.0	13.9	27.6
April	7,806	6.6	4.8	1.6	2.8	15.8
May	17,466	17.8	54.3	1.6	2.9	76.6
June	4,449	16.8	27.9	5.6	10.5	60.8
July	9,752	3.6	27.3	5.3	19.5	55.7
August	13,636	5.8	21.4	4.1	39.4	70.7
September	6,617	5.5	26.2	5.8	33.2	70.7
October	10,548	2.0	22.6	12.4	7.4	44.4
November	9,160	3.0	18.9	6.5	20.1	48.5
December	1,609	4.7	14.0	2.4	30.3	51.4

The total parasitism by each species in all fruits as recorded in Table 5 definitely shows the efficiency of each parasite and the total parasitism by all four species from 1915 to 1921, inclusive. *Opius humilis* has declined steadily in effectivenesss since 1915, when it parasitized 31.5 per cent of *Ceratitis capitata* in all fruits until 1921, when its parasitism was only 7.6 per cent. From a position of first importance in 1915 and 1916, it has been reduced in numbers until it ranked third in effectiveness in 1920 and 1921. As previously recorded (*3*, *5*), this decrease is the result of the certain destruction of *O. humilis* by either *Diachasma tryoni* or *Diachasma fullawayi* when they occur in the same host larva. The two species of Diachasma will probably never eliminate *O. humilis* from Hawaii, but they will doubtless decrease its numbers to such an extent that it will be the least effective of any of the four parasites, and a very small factor in the killing of *C. capitata*. It has been maintained as a result of previous investigations (*3*, *5*) that *O. humilis*, being more prolific and capable of destroying a higher percentage of *C. capitata* under Hawaiian conditions than either species of Diachasma, would be more effective, working alone, than *O. humilis*, *D. tryoni*, and *D. fullawayi* combined. The steady decrease of *O. humilis*, from a place of first to one of very little importance, supports this contention

TABLE 5.—*Total parasitism of all larvae of Ceratitis capitata collected in Hawaii from 1915 to 1921, inclusive*

Year	Number of larvae	Percentage of parasitism				
		Opius humilis	Diachasma tryoni	Diachasma fullawayi	Tetrastichus giffardianus	Total
1915	28,010	31.5	0.3	5.9	0.2	37.9
1916	83,304	17.2	13.3	2.1	.6	33.2
1917	72,139	12.7	20.3	7.3	7.2	47.5
1918	63,480	12.4	34.6	2.6	6.2	55.8
1919	75,406	9.4	19.6	1.6	7.6	38.2
1920	57,406	9.4	22.7	12.1	7.7	51.9
1921	88,616	7.6	26.9	5.5	16.4	56.4

Diachasma tryoni is shown in Table 5 to have been the most effective parasite since 1917, and was responsible for 47.7 per cent of the total parasitism in 1921. *Tetrastichus giffardianus* has been increasing slowly in value until in 1921 its effectiveness was more than double that of any previous year, and it had advanced to second place in importance. No particular reason for the sudden increase of this species in 1921 has been observed, but it was probably the result of favorable host and climatic conditions.

SUMMARY

The infestation of fruits in Hawaii by *Ceratitis capitata*, and the work of the four introduced species of parasites during 1921, have not been to any great extent different from what they were in the four previous years. There are, however, a few notable points which have been brought out in these records.

The average infestation per fruit in 1921 was higher than usual in a number of host fruits, but this condition was accompanied by the highest yearly parasitism on record.

Percentage of parasitism by *Opius humilis* continues to decrease, and this parasite is still in third place, from the standpoint of parasitism over yearly periods; and *Tetrastichus giffardianus* has advanced from fourth to second place.

The average yearly parasitism by all four parasites for the 5-year period 1917–1921 has been approximately 50 per cent. This destruction of one-half of all *Ceratitis capitata* about Honolulu has kept down the number of adults, so that the less preferred host fruits of commercial value are harvested with little or no infestation, if removed from the trees as early as possible without impairing their value.

LITERATURE CITED

(1) Back, E. A., and Pemberton, C. E.
1915. Parasitism among the larvæ of the Mediterranean fruit fly (C. capitata) in Hawaii during 1914. Hawaii Bd. Commrs. Agr. and Forestry [Bien.] Rpt. 1913/14: 153–161.

(2) ——— and Pemberton, C. E.
1916. Parasitism among the larvæ of the Mediterranean fruit fly (C. capitata) in Hawaii during 1915. Jour. Econ. Ent. 9: 306–311.

(3) ——— and Pemberton, C. E.
1918. The Mediterranean fruit fly in Hawaii. U. S. Dept. Agr. Bul. 536, 119 p., illus.

(4) Pemberton, C. E., and Willard, H. F.
1918. Fruit-fly parasitism in Hawaii during 1916. Jour. Agr. Research 12: 103–108.

(5) ——— and Willard, H. F.
1918. Interrelations of fruit-fly parasites in Hawaii. Jour. Agr. Research 12: 285–296, illus.

(6) ——— and Willard, H. F.
1918. Work and parasitism of the Mediterranean fruit fly in Hawaii during 1917. Jour. Agr. Research 14: 605–610.

(7) ——— and Willard, H. F.
1918. A contribution to the biology of fruit-fly parasites in Hawaii. Jour. Agr. Research 15: 419–466, illus.

(8) Willard, H. F.
1920. Work and parasitism of the Mediterranean fruit fly in Hawaii during 1918. Jour. Agr. Research 18: 441–446.

(9) ———
1923. Work and parasitism of the Mediterranean fruit fly in Hawaii during 1919 and 1920. Jour. Agr. Research 25: 1–7.

A HOMOGENEOUS CARBON DISULPHIDE EMULSION[1]

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INTRODUCTION

Carbon disulphide, emulsified in water by means of soaps, is now being used throughout the area infested by the Japanese beetle, *Popillia japonica* Newm., to destroy the immature stages of this insect in the soil. All of these concentrated carbon disulphide-water emulsions tend to stratify into two or more layers of different composition which must be agitated to form homogeneous mixtures.

If a portion of such a concentrated emulsion were diluted without agitation and applied to the soil about the roots of plants, the dilution made from the heavier layer would have an excessive proportion of the insecticide and would be likely to injure the plants, while the dilution made from the lighter layer might not be strong enough to kill the insect. Experience has shown that these concentrated carbon disulphide-water emulsions were not entirely satisfactory, owing to the variability in the concentration of the dispersed phase, and experiments were conducted to make an emulsion of carbon disulphide which would not have this undesirable property.

EXPERIMENTAL WORK

As all of these emulsions contain two immiscible liquids—carbon disulphide and water—it was thought that a more satisfactory concentrated emulsion could be made by substituting for the water some other liquid which is more miscible with carbon disulphide. Very good concentrated mixtures of this type were made by dissolving the carbon disulphide in methyl or ethyl alcohol. The dilute emulsions were made by precipitating the carbon disulphide from the alcoholic solution by the addition of water. This procedure, however, which was used in the case of certain oils by Lewis (*6*) and by Joshi (*5*), is effective only when there is a very small proportion of carbon disulphide in the alcoholic mixture. Since it is desirable to have a large proportion of carbon disulphide in these mixtures, it was thought necessary to add a suitable third substance.

Investigators have observed that the addition of 5 per cent methyl alcohol to mixtures of ethyl alcohol and petroleum (*1*) increased the stability of the mixtures when diluted with water. Transparent homogeneous emulsions can be made by adding glycerol to a solution of calcium oleate in carbon tetrachloride (*2, p. 30*). A solution of a cresol soap (*4*) has been prepared from linseed oil, potassium hydroxide, alcohol, water, and crude cresol, and a liquid soap with formaldehyde (*3*) has been prepared from castor oil saponified with alcoholic

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potassium hydroxide, water, and formaldehyde; but, so far as the writer has been able to determine, no concentrated mixture of carbon disulphide has been prepared with alcoholic potassium hydroxide and oleic acid.

The preliminary experiments for determining the nature of the third substance to be added to the carbon disulphide-alcohol mixture indicated that a soap is the best addition for increasing its stability with water. The sodium soap formed from oleic acid and different proportions of sodium hydroxide were not sufficiently miscible with carbon disulphide to be satisfactory, but the potassium soaps formed in the same manner mixed well with carbon disulphide and alcohol.

The next step was to determine the maximum volume of carbon disulphide and the optimum quantities of alcohol, oleic acid, and potassium hydroxide to be used. Alcoholic potassium hydroxide[2] was mixed with U. S. P. oleic and 95 per cent ethyl alcohol, in the proportions of hydroxide 42 c. c., oleic acid 48.5 c. c., and ethyl alcohol 8 c. c. This mixture contained about 2 per cent excess of oleic acid over the quantity required to react with the potassium hydroxide to assure complete conversion of the potassium to potassium oleate, and thus prevent the loss of carbon disulphide by the action of potassium alcoholate, converting it to potassium xanthate. This alcoholic potassium oleate was mixed with carbon disulphide in the proportions of 1:9, 2:8, 3:7, 4:6, and 5:5, by volume. Although the lower proportions of the alcoholic potassium oleate gave concentrated mixtures having the best appearance, there was not sufficient soap to disperse the carbon disulphide in water. The mixture 2:8 seemed to have the minimum proportion of soap necessary to keep the mixture from disintegrating when diluted with water.

The results having indicated that 70 per cent was near the maximum proportion of carbon disulphide which should be put into the mixture, the percentage of excess ethyl alcohol was varied from 0 to 22, and the corresponding percentage of alcoholic potassium oleate from 30 to 8. Each of these 70 per cent carbon disulphide emulsions was diluted by pouring 5 c. c. of water into 5 c. c. of the emulsion, and, after slight agitation, adding it to a large volume of water. It was not possible to produce a homogeneous dilution by pouring any of these emulsions directly into a large volume of water, but it was possible to obtain such dilutions after the initial dilution obtained by adding an equal volume of water to the concentrated emulsion. These mixtures made good dilutions with water, the stability of the dilution being proportionate to the amount of soap present. The results of these different mixtures, which are outlined in Table 1, indicate that the mixture containing 14 per cent ethyl alcohol, 8.56 per cent oleic acid, and 7.44 per cent potassium alcoholate was the best. Expressed in other terms, the composition of a liter of the best alcoholic carbon disulphide emulsion is:

C. P. carbon disulphide	700.00 c. c.
95 per cent ethyl alcohol	214.40 c. c.
U. S. P. oleic acid	85.60 c. c.
U. S. P. potassium hydroxide	14.88 gm.

[2] Alcoholic potassium hydroxide (potassium alcoholate) was prepared by refluxing 100 gm. of U. S. P. potassium hydroxide with 400 c. c. of 95 per cent ethyl alcohol, and, after cooling, filtering the supernatant liquid through a dry filter. The alcoholate was standardized by titrating a diluted aliquot part against 0.1/N hydrochloric acid, using methyl orange as an indicator, and so adjusted with 95 per cent ethyl alcohol as to contain 0.2 gm. of potassium hydroxide per cubic centimeter.

TABLE 1.—*Characteristics of insecticidal emulsions and their stability when diluted; composed of 70 per cent of carbon disulphide and various proportions of alcohol, oleic acid, and potassium alcoholate*

Proportions of—			Characteristics of concentrate	Stability of dilution, 5 : 100 of water
Ethyl alcohol (95 per cent)	Oleic acid	Potassium alcoholate		
Per cent	*Per cent*	*Per cent*		
0	16.05	13.95	Considerable flocculent precipitate	Very good.
2	14.98	13.02	do	Very good.
4	13.91	12.09	Flocculent precipitate	Very good.
6	12.84	11.16	do	Very good.
8	11.77	10.23	do	Good.
10	10.70	9.30	Slight flocculent precipitate	Good.
12	9.63	8.37	Transparent amber solution	Good.
14	8.56	7.44	do	Good.
16	7.49	6.51	do	Fair.
18	6.42	5.58	do	Fair.
20	5.35	4.65	do	Fair.
22	4.28	3.72	do	Poor.

ADDITION OF OILS OR FATTY ACIDS

Further experiments were made to determine whether the dispersion of the carbon disulphide in water could be facilitated by the addition of oils or fatty acids to the concentrated emulsion. Mixtures were prepared containing 70 per cent carbon disulphide and different proportions of alcoholic potassium oleate (95 per cent ethyl alcohol, 214 c. c.; U. S. P. oleic acid, 86 c. c.; and U. S. P. potassium hydroxide, 15 gm.), together with coconut oil, cottonseed oil, copaiba oil, linseed oil, olive oil, oleic acid, or lactic acid. It was found that satisfactory concentrated mixtures could be made with coconut oil, cottonseed oil, linseed oil, and olive oil. Of these, the mixture in which 10 per cent of the alcoholic potassium oleate was replaced by cottonseed oil appeared to be the most efficacious in facilitating the dispersion of the carbon disulphide in water. The formula of the carbon disulphide emulsion was therefore modified to be as follows:

C. P. carbon disulphide	700 c. c.
U. S. P. oleic acid	77 c. c.
Ethyl alcohol (95 per cent)	193 c. c.
Cottonseed oil (purified)	30 c. c.
U. S. P. potassium hydroxide	13.5 gm.

To prepare a liter of this alcoholic emulsion, dissolve an excess of potassium hydroxide in alcohol; then filter off the insoluble carbonate and, after determining the hydroxide content by titration against a standard acid, add sufficient alcohol to obtain a concentration of 13.5 gm. of potassium hydroxide in 193 c. c. of alcohol; then pour 77 c. c. of oleic acid into each 193 c. c. of the alcoholic potassium hydroxide solution; then add 700 c. c. of carbon disulphide and 30 c. c. of cottonseed oil to each 270 c. c. of the oleic acid-alcoholic solution.

The resulting emulsion is amber colored, transparent, and homogeneous over a relatively long period. It is readily measured in small quantities, and mixes well with water. It must be diluted initially with an equal volume of water before being mixed with the larger quantity of water used in the insecticidal treatment in order to obtain a good dispersion of the carbon disulphide.

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The resulting emulsion is amber colored, transparent, and homogeneous over a relatively long period. It is readily measured in small quantities, and mixes well with water. It must be diluted initially with an equal volume of water before being mixed with the larger quantity of water used in the insecticidal treatment in order to obtain a good dispersion of the carbon disulphide.

SUMMARY

A concentrated carbon disulphide emulsion has been prepared for insecticidal use which is transparent, homogeneous, and readily measured in small quantities. It is necessary to pour an equal volume of water into the emulsion and agitate it before adding it to the larger volume of water used in the insecticidal treatment. The composition of this emulsion is given.

LITERATURE CITED

(1) AUBERT, M., and DIXMIER, G.
1923. STABILITÉ DES MÉLANGES ALCOOL-ESSENCE EN PRÉSENCE DE L'EAU. Compt. Rend. Acad. Sci. [Paris] 176: 1307–1310, illus.

(2) CLAYTON, W.
1923. THE THEORY OF EMULSIONS AND EMULSIFICATION. 160 p., illus. London.

(3) CORDONNIER, E.
1922. SAVON LIQUIDE AU FORMOL. Bul. Sci. Pharmacol. 29: 559–562.

(4) FREYMUTH, A.
1922. [SOLUTION OF CRESOL SOAP.] Deut. Parfüm. Ztg. 8: 28–30. [Not seen. Abstract in Chem. Abs. 17: 613. 1923.]

(5) JOSHI, S. S.
1924. DIE OBERFLÄCHENSPANNUNG VON OEL-IN-WASSER- UND WASSER-IN-OEL-EMULSIONEN. I. Kolloid Ztschr. 34: 197–201.

(6) LEWIS, W. C. McC.
1909. GRÖSSE UND ELEKTRISCHE LADUNG DER OELTEILCHEN IN OEL-WASSER-EMULSIONEN. Ztschr. Chem. u. Indus. Kolloide 4: 211–212.

CARDIOCHILES NIGRICEPS VIER., AN IMPORTANT PARASITE OF THE TOBACCO BUD WORM, HELIOTHIS VIRESCENS FAB.[1]

By F. S. CHAMBERLIN, *Assistant Entomologist*, and J. N. TENHET, *Junior Entomologist, Southern Field-Crop Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The most effective insect enemy of the tobacco bud worm, *Heliothis virescens* Fab., in the southern tobacco region[2] of the United States is the ichneumon fly, *Cardiochiles nigriceps* Vier.

While studying the biology and control of various tobacco insect pests at Quincy, Fla., the writers made observations and notes on the life history and habits of this parasite. The present paper summarizes the data collected.

HISTORY

Cardiochiles nigriceps was first described in 1912, by Viereck.[3] In 1917 Morgan and McDonough[4] made brief mention of the economic importance of this bud-worm parasite under the name *Toxoneura* sp. Aside from these two references, no mention has been found in the literature regarding this insect.

ECONOMIC IMPORTANCE

From the standpoint of economic importance, *Cardiochiles nigriceps* stands easily first among several species of parasites that attack the tobacco bud worm in the tobacco-growing sections of Georgia and Florida. Several seasons' observations would indicate that it remains fairly constant in abundance from year to year.

The economic value of this parasite is very difficult to determine, owing to the high mortality of young bud worms under laboratory conditions. It appears, however, that many young bud-worm larvae die very quickly as the result of its attack, although the majority approach maturity before they succumb. As the tobacco bud-worm larva continues to do serious damage to tobacco buds throughout its entire period of growth, it is evident that the benefit derived from *Cardiochiles nigriceps* is more cumulative than immediate.

During the early part of the tobacco-growing season the parasite is observed only rarely, but as the season advances its numbers rapidly increase. In July and August parasitism by this species frequently averages from 50 to nearly 100 per cent.

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[1] Received for publication Dec. 15, 1925; issued July, 1926.

[2] Northern Florida and southern Georgia.

[3] VIERECK, H. L. DESCRIPTIONS OF ONE NEW FAMILY, EIGHT NEW GENERA, AND THIRTY-THREE NEW SPECIES OF ICHNEUMON-FLIES. U. S. Nat. Mus. Proc. 43: 575-593. 1912.

[4] MORGAN, A. C., and MCDONOUGH, F. L. THE TOBACCO BUD WORM AND ITS CONTROL IN THE SOUTHERN TOBACCO DISTRICTS. U. S. Dept. Agr. Farmers' Bul. 819, 12 p., illus. 1917.

All attempts to breed *Cardiochiles nigriceps* from hosts other than *Heliothis virescens* have yielded negative results; nor has it been reported as parasitic on other insects. It appears, however, to attack the bud worm on its various food plants other than tobacco. The percentage of parasitism on beggarweed (*Meibomia* sp.), which is the favored food plant of the tobacco bud worm during the early fall months, is usually much less than on tobacco at the same season. This is apparently due to the fact that beggarweed is scattered over

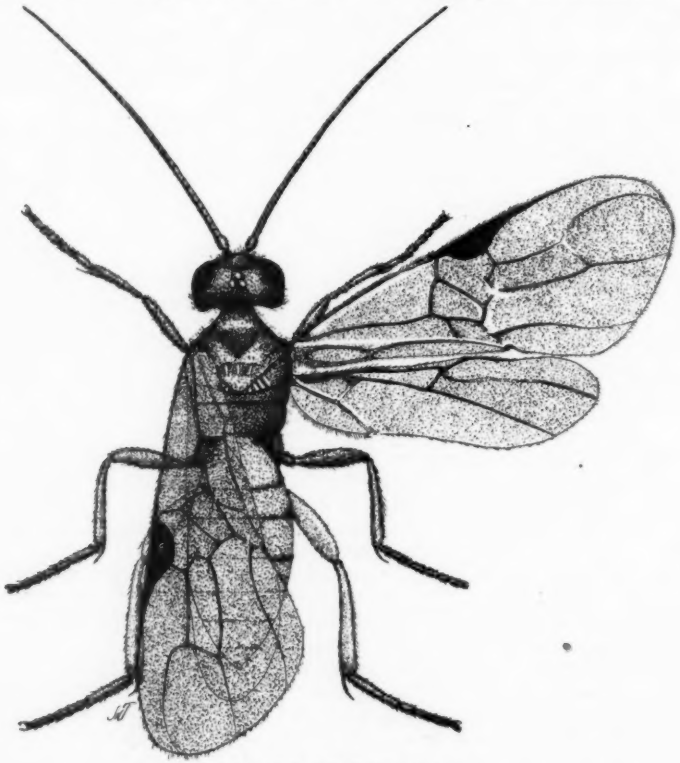

FIG. 1.—Adult of *Cardiochiles nigriceps*

great areas of land, whereas tobacco is concentrated on relatively small areas. If the last brood of bud worms in the fall confined its attack to tobacco suckers, the benefit derived from *Cardiochiles nigriceps* would doubtless be much greater.

DISTRIBUTION

It appears that *Cardiochiles nigriceps* is limited to the southeastern part of the United States. Many observations show that it is abundant in the Georgia and Florida tobacco-growing sections. It has also been reported from Alabama and Mississippi, and has been found as far north as Huntsville, Ark., and Clarksville, Tenn.

CLASSIFICATION AND DESCRIPTION

Cardiochiles nigriceps is a member of the hymenopterous superfamily Ichneumonoidea and family Braconidae. The adult (fig. 1) has been well described by Viereck,[5] as follows:

Type-locality.—Savannah, Georgia.

Type.—Cat. No. 15007, U.S.N.M.

Cardiochiles nigriceps, new species. *Female.*—Length 7.4 mm.; head including antennae and palpi, prescutum, scapulae, pleurae, wings, coxae, trochanters, fore and mid femora, hind tarsi, tips of hind tibiae and propodeum mostly black or blackish, elsewhere, excepting fore and mid tibiae and tarsi which are dark brown or blackish, mostly reddish. Related to *C. viator* (Say) and *C. seminigra* (Cresson).

Allotype.—Essentially as in the type.

Labeled, "*Toxoneura nigriceps* Riley. No. 3956,° reared Aug. 10, 1886, from *Chloridea rhexia*, received from Mr. R. S. Barnoll."

A series of female paratypes labeled "Am. Sumatra Tobacco Co.," from Quincy, Florida, shows variation in color, one specimen in particular having the prescutum and scapulae almost entirely reddish and the mid femora with a reddish stain. These departures from the typical specimens would seem to indicate that this species may prove to be a race of *C. viator* (Say) or *C. seminigra* (Cresson).

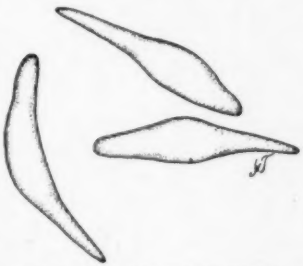

FIG. 2.—Eggs of *Cardiochiles nigriceps*

THE EGG

The egg (fig. 2) just before deposition, is slender, elongate, sharply pointed at one end and more bluntly pointed at the other; often slightly curved, decidedly thickened in the middle, and translucent white. The average length is about 0.57 mm. and the average greatest diameter is 0.11 mm.

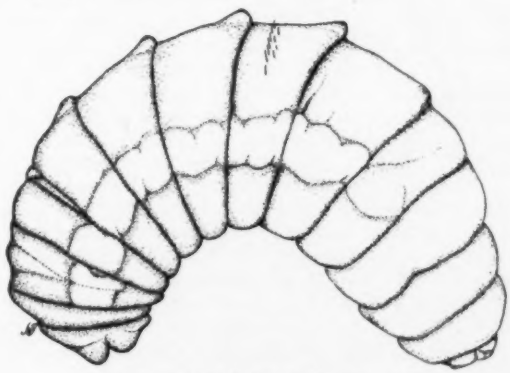

FIG. 3.—Larva of *Cardiochiles nigriceps*, lateral view

THE LARVA

The mature larva (fig. 3), as it emerges from the host, is pale yellowish green. When viewed dorsally it appears elliptical, slightly broader posteriorly. Viewed laterally, it appears more or less cres-

[5] VIERECK, H. L. Op. cit.

cent shaped, with a distinct, submedian dorso-ventral muscular ridge extending over all but a few of the segments at caudal and cephalic extremities. The well-defined body segments are 13 in number. The head is almost entirely retracted and is not chitinized; the mandibles are small and practically invisible. The average dimensions are approximately 9.4 mm. in length, when extended, and 3.4 mm. in depth, dorso-ventrally.

THE PUPA

The pupa (fig. 4) at first is creamy white in color, and resembles the adult in shape. The appendages are closely folded about the body. The pupa gradually darkens, until the characteristic adult colors appear and can be dimly seen through the silken-walled cocoon.

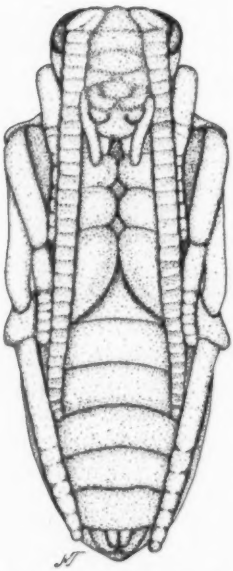

FIG. 4.—Pupa of *Cardiochiles nigriceps*, ventral view

LIFE HISTORY

THE EGG PERIOD

All attempts to dissect the egg of *Cardiochiles nigriceps* from the body of its tiny host, or to ascertain the exact length of the incubation period, have been unsuccessful.

The period of oviposition usually begins about the middle of April, when the young tobacco plants are just becoming established in the fields, and continues until about the middle of September, when young bud worms are becoming scarce.

THE LARVAL PERIOD

As it proved impossible to ascertain the time when the egg hatched, the beginning of the larval period could not be determined. The combined incubation and larval periods (including the prepupa), however, ranged from about 17 to 21 days in midsummer.

The larva feeds within the body of its host, developing as the bud worm develops, until maturity is reached. The host seldom succeeds in growing to normal size, but usually enters the soil when apparently only about two-thirds grown. The parasitized bud worm never succeeds in pupating. When fully mature the parasite eats a small hole through the dorsal or lateral surface of its host, and slowly emerges. Nothing but the empty skin of the bud worm is left. All records indicate that only one parasite can complete its development in each host larva.

Shortly after emergence, the larva begins to spin a cocoon. This cocoon is made by the larva turning around and around in the soil, forming a cell and spinning a tight silken wall. The cocoon can be formed only underground. When the larva is placed on top of the earth it produces a mass of silk, but is unable to complete a cocoon.

THE COCOON

The cocoon is broadly ellipsoidal in shape, dirty white, and very tough. In general aspect it rather closely resembles the cocoons of some of the larger Lepidoptera. In size it is usually 8 to 9 mm. long and 3 to 4 mm. in diameter.

THE PREPUPAL PERIOD

After completion of the cocoon there is an interval of approximately four days before pupation takes place. This interval must be considered as a prepupal stage, in which the larva is gradually contracting and preparing to pupate.

THE PUPAL PERIOD

It has been determined by repeated dissection of cocoons that pupation takes place on or about the fourth day after the cocoon is formed. During the summer the length of the pupal period usually ranges from 8 to 12 days.

THE ADULT PERIOD

Just how long the adult insect lives under normal conditions seems never to have been established. In the laboratory very few were kept alive more than four or five days. When caged, they strive continually to escape, and all efforts to induce feeding, copulation, or oviposition failed.

HABITS

OVIPOSITION

When the weather is bright and warm, *Cardiochiles nigriceps* may be observed in tobacco fields at almost any hour of the day. Ovipositional activity is much greater in the middle of the day. In cool, cloudy weather the parasite appears to be sluggish, and seeks concealment and protection beneath tobacco leaves, weeds, or débris.

The female parasite prefers as its victims bud worms in the first instar, although oviposition frequently occurs in second-instar larvae. Only one instance has been observed where an egg was apparently deposited in a larva of the third instar. Large bud worms are ignored.

The adult flies slowly from plant to plant, hovering about each one for some little time. Frequently the search seems to be made quite systematically. The insect goes carefully over the entire upper portion of the plant, usually keeping about an inch from the leaf surface. It gives special attention to the buds and suckers. The tiny bud worm appears to be located by the senses of touch and smell. Often the parasite will alight and examine some particular spot more closely, feeling carefully with her antennae. Frequently, if there are signs of fresh eating on the leaf, she seems to become much excited. The ovipositor is extruded, and the insect makes tentative stabs as though trying to locate the missing bud worm. Often she will walk over a bud, exploring it carefully with her antennae and thrusting her ovipositor into every crack and fold in an attempt to reach the tiny larva in the center of the bud.

When a bud worm is located, the parasite evinces signs of intense excitement. She taps the tiny worm all over with her antennae, sometimes even knocking it away in her movements. When she has determined the exact position of the larva, she straddles it, and, extruding her ovipositor, attempts to thrust it into her victim. Sometimes she repeatedly misses it and has to turn apparently to relocate the worm. The ovipositor is thrust either into the dorsal or lateral surface of the larva, usually about the middle, but quite frequently near the caudal or the cephalic end. The act of oviposition requires only about two or three seconds. As soon as the ovipositor is withdrawn the parasite immediately begins searching for another victim.

There appears to be a marked tendency on the part of *Cardiochiles nigriceps* to avoid ovipositing in a bud worm which has previously been parasitized. A female will frequently return to a tobacco plant which she has left only a short time before, but she ignores the larvae which she parasitized on her former visit. Very often a female will refuse to oviposit in young bud worms of the preferred size, and it is inferred that they have previously been parasitized.

For two or three minutes after an egg is deposited, the bud worm appears to be completely paralyzed. When it is noted that the inserted egg is often from one-third to one-half the length of the larva, this is not surprising. In a short time, however, the bud worm begins feeding again.

OTHER BEHAVIOR

As already mentioned, the present writers have no knowledge of *Cardiochiles nigriceps* ever having been bred from any insect other than *Heliothis virescens*. In two instances, however, this parasite appeared to attempt oviposition in first-instar tobacco hornworms (*Protoparce sexta* Joh.). In each instance the hornworms were reared to the pupal stage, but no signs of parasitism developed. It is very unlikely that the hornworm can serve as a host for this parasite.

Numerous adults of *Cardiochiles nigriceps* have been collected in tobacco fields, and examinations have shown that the sexes are about equally represented, but the writers have never observed copulation.

Although the adult insect manifests great excitement under cage conditions, in the field it shows no fear of man. A female will work undisturbed with an observer standing close beside the tobacco plant. The stalk upon which she is resting may even be bent downward for closer observation without disturbing her.

SEASONAL HISTORY

In the vicinity of Quincy, Fla., adults of *Cardiochiles nigriceps* appear in the spring soon after tobacco has been set in the fields, usually from the first to the middle of April. By May they are usually observed in considerable numbers, and from then until the middle of September they are very abundant. After the middle of September the numbers decline, although specimens have been observed in the field as late as November 3. Emergence of adults in outdoor cages has been recorded from March 20 until September 11.

There are probably three or four generations a year in this locality, but the last two are much the largest. In July and August this parasite is so abundant in tobacco fields that frequently three or four may be observed flying about a single stalk of tobacco. At this season of the year 50 to nearly 100 per cent parasitism by this species is not unusual.

Cage records checked with field observations indicate that the winter is passed in the pupal stage, in the soil below the surface.

SUMMARY

Cardiochiles nigriceps is the most important insect parasite of the tobacco bud worm, *Heliothis virescens*, in the southeastern part of the United States. In July and August parasitism by this species frequently ranges from 50 to nearly 100 per cent.

As far as the writers are aware, this parasite attacks only the true bud worm of tobacco, *Heliothis virescens*.

At Quincy, Fla., the combined incubation and larval periods required from 17 to 21 days during the summer months. The pupal period ranged from 8 to 12 days.

VITAMIN A IN POULTRY FLESH AND FAT[1]

By RALPH HOAGLAND, *Biochemist, Biochemic Division*, and ALFRED R. LEE, *Associate Poultry Husbandman, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In a previous investigation [2] the writers studied the vitamin B content of poultry flesh and eggs. In this paper they report the results of experiments conducted with rats for the purpose of determining the vitamin A value of the body flesh and fat of several species of domestic fowl. These tests were made on fowls reared under ordinary conditions and fed rations which were in general use. The vitamin A content of eggs has already been reported by several investigators, but there appears to be no information in the literature concerning the amount of vitamin A in the products tested by the writers.

EXPERIMENTAL WORK

FEEDING HISTORY OF FOWLS TESTED

Since it is probable that the vitamin A content of the flesh and fat of poultry is directly related to the amount of that vitamin in the diet of the birds, it seems desirable to record briefly the feeding history of each lot of fowls tested.

Barred Plymouth Rock hens.—This lot of birds consisted of four hens which had been raised on the experiment farm of the United States Department of Agriculture at Beltsville, Md. These chickens, which were hatched in the spring of 1923, had free grass range during the growing period, and were fed a ration previously described by the writers [3] until 6 months old. The pullets were then put in laying pens with moderate-sized yards which contained a fair amount of grass during the growing season. The ration fed to the hens from the time they were 6 months old until October, 1924, consisted of scratch feed made up of equal parts of corn, wheat, and oats, and a mash containing 4 parts rolled oats 2.5 parts meat scrap, 2 parts bran, 2 parts corn meal, and 1 part middlings, to which was added 5 per cent ground bone and 1 per cent salt. In addition, during the fall and winter months the hens were given a light feed of sprouted oats or cabbage two or three times a week. From October 1 to December 15, 1924, the hens received only scratch feed, after which they were fed mash also, until killed January 13, 1925, when the average live weight of the hens was 5.5 pounds.

Rhode Island Red hens.—This lot of birds consisted of five hens, 22 months old, which had also been raised on the experiment farm at Beltsville. These hens were raised and kept under practically the same conditions as those described for the Barred Plymouth Rock hens. The average live weight of the hens when killed, January 31, 1925, was 5.5 pounds.

Muscovy ducks.—These ducks—three small females and one large male—were purchased from a farm in Beltsville. The male bird was hatched in 1923 and the females in 1924. They were fed lightly on a commercial laying mash supplemented with corn meal and whole corn, and they had free range on which there was an abundance of green feed. No additional green feed was provided during the winter months, but the ducks were able to get considerable green stuff from the range during the early part of that season. The ducks were killed February 25, 1925, the average live weight at the time being 6.3 pounds.

[1] Received for publication Nov. 19, 1925; issued July, 1926.

[2] HOAGLAND, R., and LEE, A. R. ANTINEURITIC VITAMIN IN POULTRY FLESH AND EGGS. Jour. Agr. Research 28: 461–472. 1924.

[3] HOAGLAND, R., and LEE, A. R. Op. cit. p. 463.

Pekin ducks.—These birds—3 females and 1 male—were obtained from a dealer who had purchased them from a farmer in Virginia. The ducks were hatched in the spring of 1924, but no information is available concerning their feeding on the farm. The dealer held these ducks for about a month before slaughter, during which time they received only a limited amount of green feed. The ducks were slaughtered March 7, 1925, the average live weight at the time being 4.75 pounds.

Toulouse geese.—Two female geese, approximately 2 years old, were purchased from a farmer near Beltsville. They were raised on free range, and received corn as grain. During the winter previous to slaughter they had a rye pasture and were fed a commercial dairy mash mixed with skim milk. The geese were in good flesh when slaughtered, January 15, 1925, the average live weight being 12.2 pounds.

Embden geese.—Three ganders were purchased alive on the Washington, D. C., wholesale market February 28, 1925. They had probably been raised on near-by Virginia or Maryland farms, but no information concerning their feeding history was available. The geese were killed March 2, 1925, the average live weight at the time being 9.6 pounds.

White Holland turkeys.—One male bird, approximately 21 months old, was purchased February 2, 1925, from a farm near Beltsville. The turkey had been fed a commercial scratch grain, but no mash, and had been allowed free range on which there was a fairly good amount of green feed. The live weight of the bird at the time of slaughter was 23.4 pounds.

Mammoth bronze turkeys.—Two male birds, approximately 10 months old, were purchased from a dealer in poultry who conducts a small farm near Washington, D. C. He purchased the turkeys from farmers in Virginia about November 15, 1924, and held them on his farm for about three months until they were purchased for this experiment. During this period they were allowed a very moderate range, but the amount of green feed was limited at that season of the year. The turkeys were fed only corn during this time. No information is available concerning the conditions under which the turkeys were raised on the farm. The average live weight of the turkeys when killed February 11, 1925, was 15.5 pounds.

Pearl gray guinea fowls, lot 1.—Eight young female guinea fowls, hatched in the spring of 1924, were purchased from a farmer near Hyattsville, Md. The young guinea chickens were started on a commercial chick feed and were continued on it for a few weeks, and then they were allowed free range. After that they were fed a small amount of corn and wheat, but received no other feed except what they picked up. The amount of green feed on the range during the winter months was undoubtedly very limited, but it was probably greater than that available for the Plymouth Rock and Rhode Island Reds hens from the experiment farm at Beltsville. The average live weight of the guinea hens at the time of slaughter, January 23, 1925, was 2.7 pounds.

Pearl gray guinea fowls, lot 2.—Seven male and two female guinea fowls were purchased from a dealer who had bought them from a farmer in Virginia. The dealer kept the birds on his farm for about a month after purchase, feeding them corn and allowing them free range, but the amount of green feed was limited at that time. No information was available concerning the feeding of the guinea fowls. The average weight of the birds at the time of slaughter, March 9, 1925, was 3.1 pounds.

Promptly after slaughter the muscle and fatty tissues were trimmed from the carcasses of each lot of fowls. The muscle tissue was ground, mixed with water and toluol, and dried to air dryness in a current of air at a temperature not higher than 60° C., the operation requiring less than 24 hours. The dried material was ground, transferred to glass bottles, and stored at a temperature approximating 4° C. until tested for vitamin A. The fatty tissues were rendered on a steam bath at a temperature not higher than 75° C., the operation requiring less than one hour. The rendered fat was filtered into glass jars, which were placed in cold storage at the temperature noted above. The samples of dried poultry flesh were analyzed for protein and fat and each sample of flesh and fat was tested promptly for vitamin A.

METHODS EMPLOYED IN TESTING FOR VITAMIN A[4]

The relative amounts of vitamin A in the samples of poultry flesh and fat were determined by feeding tests with young albino rats, the sample under examination constituting the only known source of this vitamin in an otherwise adequate ration. The rats were raised in the animal laboratory of the Bureau of Animal Industry. Litters containing more than 8 rats were reduced to that number on the day of birth, and those consisting of fewer than 6 were discarded. Only young rats which reached a weight of 40 grams within 30 days from birth were used for test purposes. The breeding rats were fed a ration relatively low in vitamin A.

Each sample of flesh, or fat, was mixed in one or more proportions in rations made up according to the following standard:

Basal ration

	Parts
Protein (N×6.25)	20
Ash	4
Yeast	10
Fat	20
Starch to make	100

The protein consisted of finely ground commercial casein which had been heated 72 hours in a current of air at an average temperature of 110° to 115° C. to destroy vitamin A. The ash mixture was made up according to a formula by Drummond and Watson.[5] Dried bakers' yeast, hardened cottonseed oil, and cassava starch comprised the remainder of the ration. Casein was the only constituent of the basal ration found to require purification from vitamin A.

Each ration was fed to a group of 4 rats. Each rat was kept in an individual cage with a raised-screen bottom. The ration was supplied in a self-feeder, and a record was kept of the feed consumed. The rats were weighed biweekly and they were observed frequently for signs of ophthalmia.

FEEDING TESTS WITH BASAL RATIONS

Each ration containing poultry flesh or fat was fed to a group of rats from the start, without a preliminary test on a vitamin-A-free basal ration, as is the practice followed by many investigators. The procedure which the writers have followed in this work requires that proper allowance be made for the growth made by rats fed the basal rations alone under the same conditions as those existing in the other experiments.

In Figure 1 are shown the results obtained by feeding the basal ration to two groups of rats during this investigation. The rats made but limited growth, and all but two developed ophthalmia. The growth made by these rats probably is due largely, if not entirely, to the reserve store of vitamin A in their tissues, rather than to the presence of this vitamin in the basal ration.

[4] The feeding experiments with rats were carried on by George G. Snider and Warren L. Briggs, of the Biochemic Division, Bureau of Animal Industry.

[5] DRUMMOND, J. C., and WATSON, A. F., THE TESTING OF FOODSTUFFS FOR VITAMINS. Analyst 47: 237. 1922.

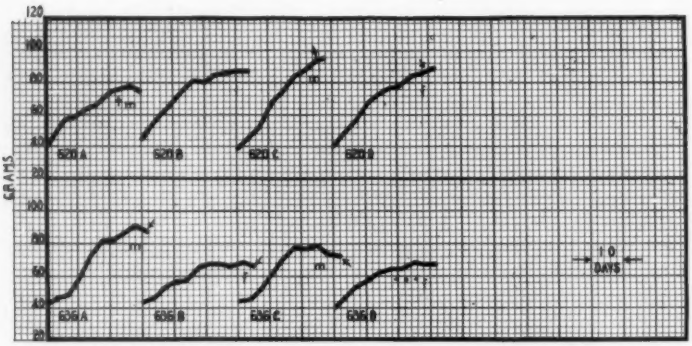

FIG. 1.—Growth of the rats which were fed the basal ration alone. Sex of rats in this and the other figures is indicated by the letters *m* and *f*; arrows indicate ophthalmia

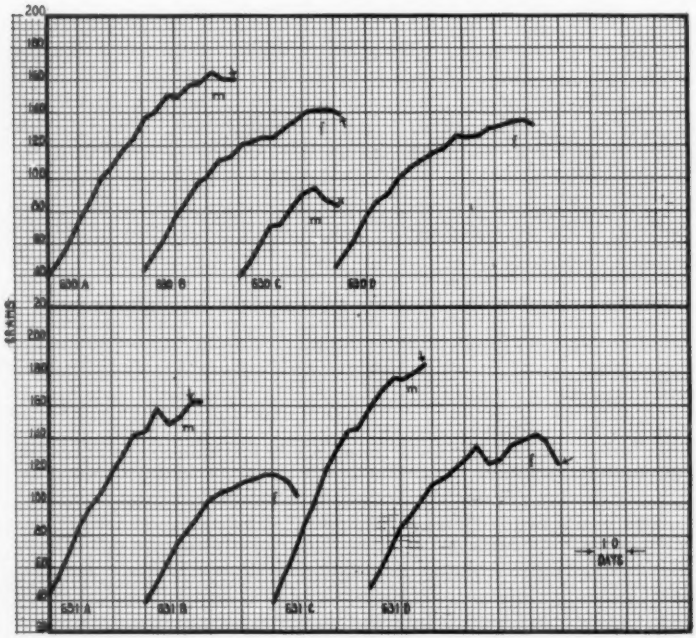

FIG. 2.—Growth of the rats fed the dried flesh of Barred Plymouth Rock hens as the source of vitamin A. The rats represented by the upper graphs were fed a ration containing 10 per cent of the flesh; and those by the lower graphs 20 per cent. Arrows indicate ophthalmia; *x* indicated severe rhinitis

VITAMIN A IN POULTRY FLESH

VITAMIN A IN CHICKEN FLESH

In Figure 2 are shown the growth curves of two groups of rats which were fed rations containing 10 and 20 per cent, respectively, dried Barred Plymouth Rock hen flesh. Rather irregular results were obtained, but by calculating the average gain in weight made by each group of rats it will be found that considerably better growth was made by those getting 20 per cent of the dried flesh. However, this proportion of the Plymouth Rock flesh did not furnish sufficient vitamin A for optimal growth.

In Figure 3 are shown the growth curves of two groups of rats which were fed rations containing 10 and 20 per cent, respectively, of dried

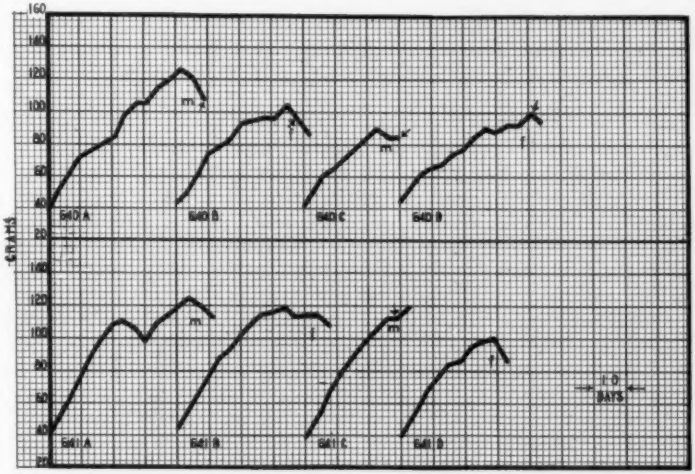

FIG. 3.—Growth of rats fed the dried flesh of Rhode Island Red hens as the source of vitamin A. Rats numbered 640 were fed a ration containing 10 per cent of the dried flesh. Those numbered 641 received a ration containing 20 per cent. Arrows indicate ophthalmia

Rhode Island Red hen flesh. The first group of rats (numbered 640) made very poor growth, and all developed ophthalmia. Although the second group of rats made somewhat better growth, it is apparent that 20 per cent of this lot of chicken flesh did not furnish nearly sufficient vitamin A for optimal growth.

On comparing Figures 2 and 3 it appears that the Barred Plymouth Rock hen flesh was appreciably richer in vitamin A than the Rhode Island Red hen flesh. Since both lots of birds were fed the same ration and handled in the same manner, it appears that the one lot of hens simply stored more vitamin A than did the other.

VITAMIN A IN DUCK FLESH

In Figure 4 are shown the results of feeding tests with the dried flesh from Muscovy and Pekin ducks, respectively. The first group of rats (numbered 657) was fed a ration containing 25 per cent of

Muscovy duck flesh, and the other group (numbered 660) received a ration containing 30 per cent of Pekin duck flesh. There is a remarkable difference in the amounts of vitamin A in these samples. All the rats getting the Muscovy duck flesh made excellent growth; in fact, rat No. 657A made extraordinary growth. On the other hand, the rats getting the Pekin duck flesh made very poor growth; three of them developed ophthalmia, and one died. It is to be noted, also,

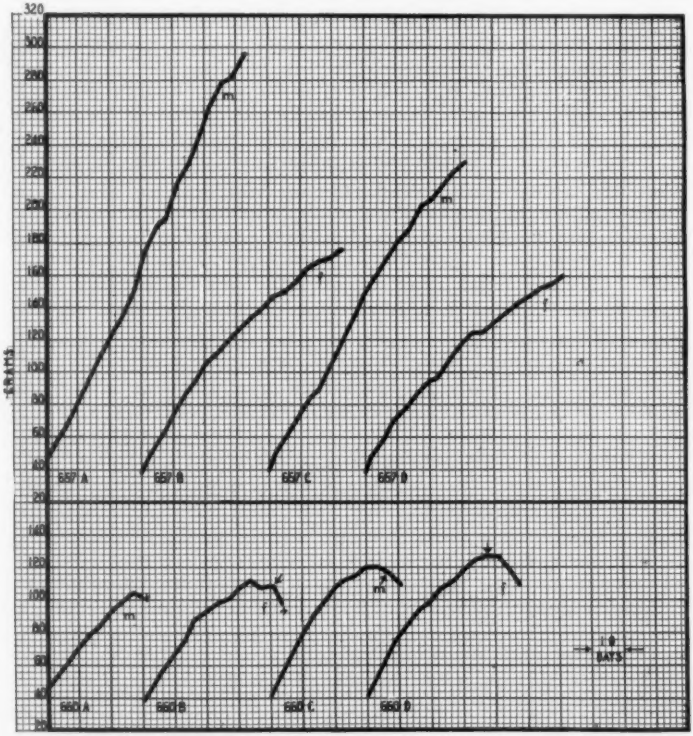

FIG. 4.—Growth of rats fed Muscovy and Pekin duck flesh as the source of vitamin A. Rats numbered 657 were fed a ration containing 25 per cent of dried Muscovy duck flesh. Those numbered 660 were fed a ration containing 30 per cent of dried flesh from Pekin ducks. Arrows indicate ophthalmia; crosses indicate death

that there was 30 per cent of Pekin duck flesh in this ration as compared with 25 per cent of Muscovy duck flesh in the other ration.

The Muscovy ducks had received an abundance of green feed in addition to mash and grain, but the Pekin ducks had only a limited amount of green feed for a month before slaughter, and information on the history of their feeding is lacking.

VITAMIN A IN GOOSE FLESH

In Figure 5 are shown the results of feeding tests with the dried flesh from Toulouse and Embden geese, respectively. Rats numbered

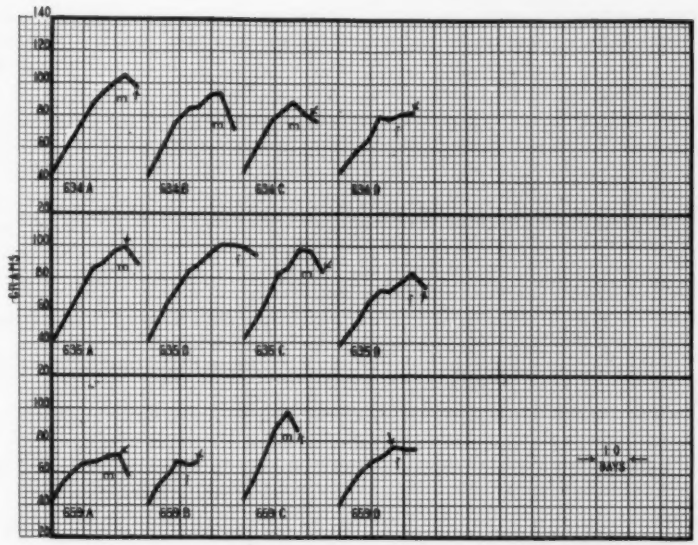

FIG. 5.—Growth of rats fed Toulouse and Embden goose flesh as the source of vitamin A. Rats numbered 634 and 635 were fed rations containing, respectively, 10 and 20 per cent of dried Toulouse flesh. Rats numbered 659 were fed a ration containing 30 per cent of Embden flesh. Arrows indicate ophthalmia

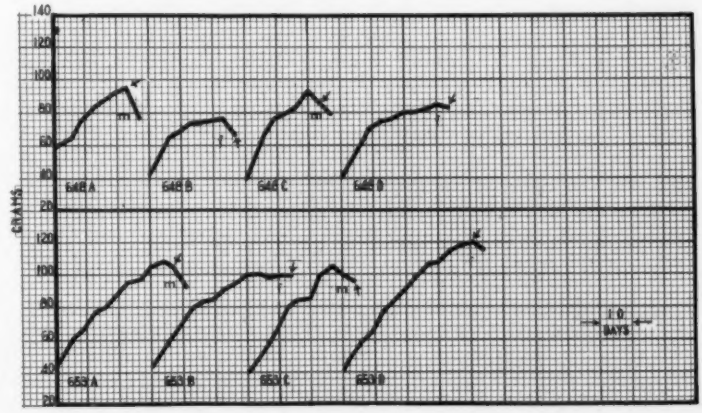

FIG. 6.—Growth of rats fed White Holland and Mammoth Bronze turkey flesh as the source of vitamin A. Rats numbered 648 were fed a ration containing 20 per cent of dried White Holland flesh. Those numbered 653 received a ration containing the same percentage of Mammoth Bronze flesh. Arrows indicate ophthalmia

634 and 635 were fed rations containing 10 and 20 per cent, respectively, of Toulouse goose flesh; and rats numbered 659 received a ration containing 30 per cent of Embden goose flesh. These graphs indicate that both lots of flesh were very poor in vitamin A, although that from the Toulouse geese appeared to contain slightly more than

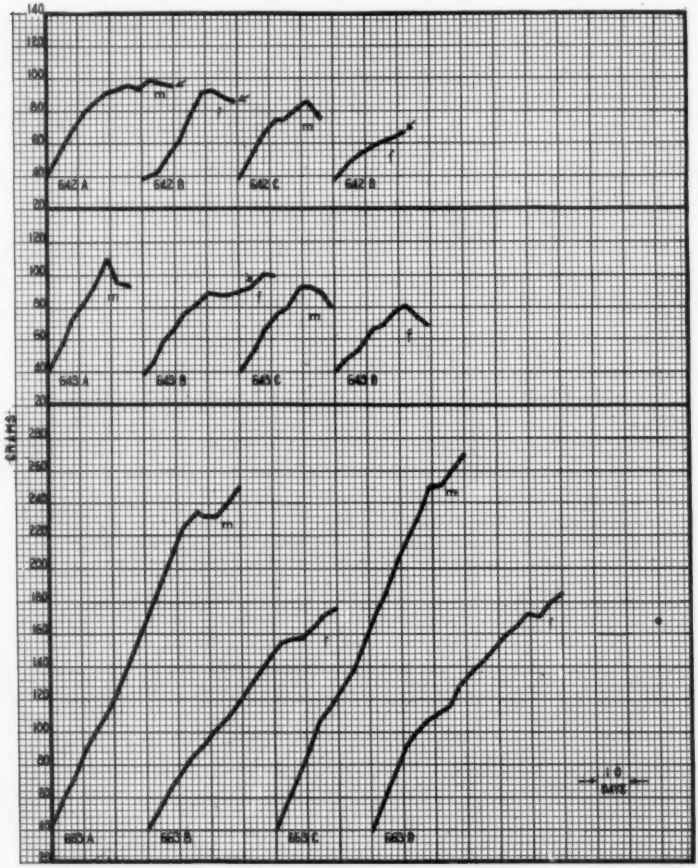

FIG. 7.—Growth of rats fed the dried flesh of guinea fowls as the source of vitamin A. Rats numbered 642 and 643 were fed rations containing, respectively, 10 and 20 per cent of flesh from the fowls purchased from a farmer near Beltsville, Md. Rats numbered 663 were fed a ration containing 30 per cent of flesh from fowls purchased from a dealer. Arrows indicate ophthalmia

than from the Embden geese. The Toulouse geese had been grown on free range with plenty of green feed, but the Embden geese were purchased from a dealer and no information concerning their feeding history was available.

VITAMIN A IN TURKEY FLESH

The results of the feeding tests with two samples of Turkey flesh are shown in Figure 6. Rats numbered 648 were fed a ration con-

taining 20 per cent of flesh from the White Holland turkey, and rats numbered 653 received an equal proportion of flesh from the Mammoth Bronze turkey. Apparently the latter sample of flesh contained slightly more vitamin A, but both samples were very poor in it.

VITAMIN A IN FLESH FROM GUINEA FOWLS

In Figure 7 are shown the results of feeding tests with the dried flesh from two lots of guinea fowls. Rats numbered 642 and 643 were fed rations containing 10 and 20 per cent, respectively, of the flesh

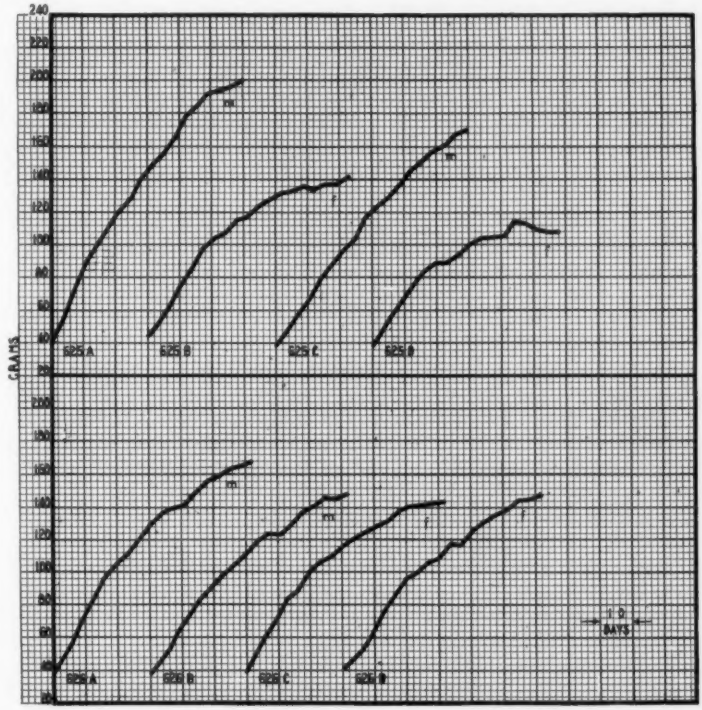

FIG. 8.—Growth of rats which received fat from Barred Plymouth Rock hens. The two groups of rats numbered 625 and 626 were fed rations containing, respectively, 5 and 10 per cent of the chicken fat

from the guinea fowls purchased from a farmer near Beltsville, and rats numbered 663 were fed a ration containing 30 per cent of the flesh from the other lot of guinea fowls which were purchased from a dealer. It is apparent from the growth curves of the rats that the first lot of guinea-fowl flesh was very poor in vitamin A. On the other hand all of the rats that were fed the second sample of flesh made remarkably good growth, and it is probable that a considerably smaller percentage of the flesh would have induced optimal growth. Although the greatest percentage that was fed of the first lot of guinea flesh was 20 per cent, whereas the second lot of flesh was fed to the

extent of 30 per cent of the ration, the difference in the growth made by the rats fed the two rations is so pronounced that it seems safe to conclude that the second sample of flesh was considerably richer in vitamin A than the first sample.

The guinea fowls purchased from a farmer near Beltsville had free range and plenty of green feed, but no information was available concerning the feeding of the other lot during the growing period. Apparently, however, the second lot of guinea fowls must have been fed a ration richer in vitamin A or had a much better range than the first lot, as indicated by the wide difference in the amounts of vitamin A in the two samples of flesh.

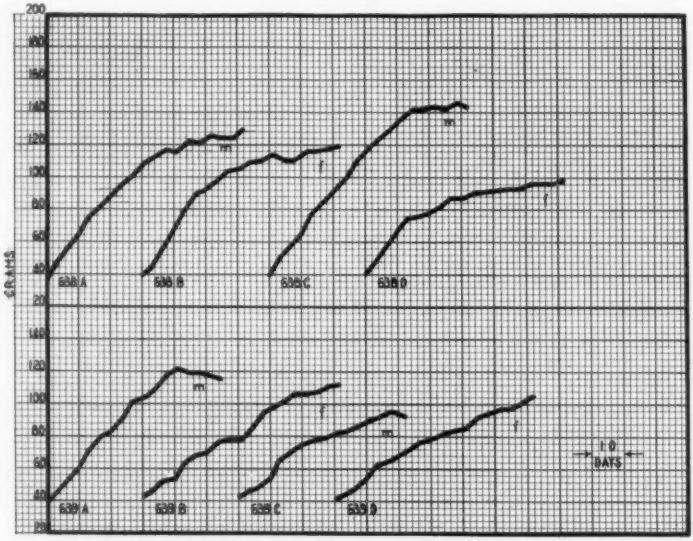

FIG. 9.—Growth of rats which received fat from Rhode Island Red hens as the source of vitamin A. Rats numbered 638 and 639 were fed rations containing, respectively, 5 and 10 per cent of the chicken fat

VITAMIN A IN POULTRY FAT

VITAMIN A IN CHICKEN FAT

The results of feeding test with rations containing fat from the Barred Plymouth Rock hens are shown in Figure 8. Although rats numbered 625 received only 5 per cent of the chicken fat, and rats numbered 626 received 10 per cent, yet the two groups made practically the same average gain in weight. Growth was considerably below normal.

In Figure 9 are shown the growth curves of two groups of rats fed rations containing 5 and 10 per cent, respectively, of fat from Rhode Island Red hens. Strangely, the rats receiving the larger proportion of chicken fat made the poorer growth. Comparing Figures 8 and 9, it seems that the fat from the Plymouth Rock hens was appreciably richer in vitamin A than that from the Rhode Island Red hens. A similar relation was previously noted concerning the amounts of

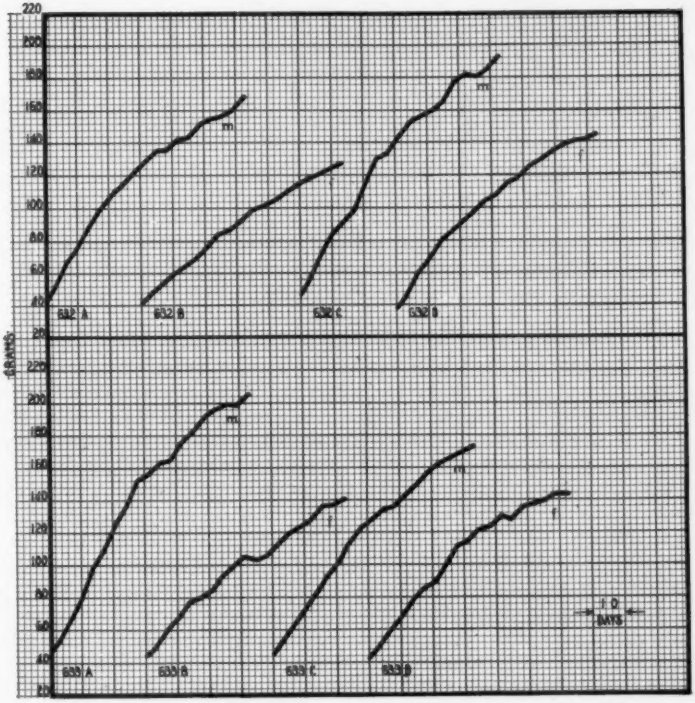

FIG. 10.—Growth of rats which received fat from Toulouse geese as the source of vitamin A. Rats numbered 632 and 633 were fed rations containing, respectively, 5 and 10 per cent of this fat

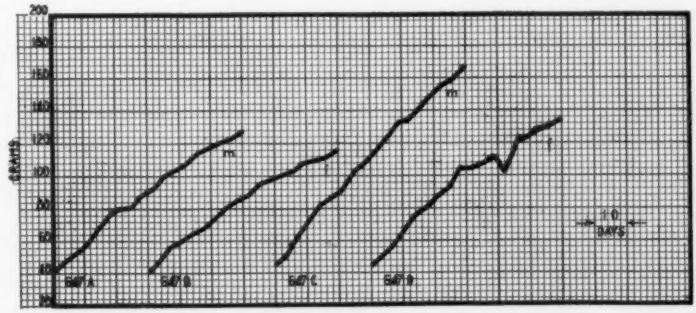

FIG. 11.—Growth of rats which received fat from White Holland turkeys. These rats were fed a ration containing 10 per cent of this fat

vitamin A in the flesh from the same two lots of hens. It is interesting to note that each lot of chicken fat was considerably richer in vitamin A than the corresponding lot of flesh (see figs. 2 and 3).

VITAMIN A IN GOOSE FAT

In Figure 10 are shown the results of feeding tests with rations containing 5 and 10 per cent, respectively, of fat from the Toulouse geese. Both groups of rats made fairly good, though not quite normal, growth, the rats getting 10 per cent of goose fat making a slightly greater average gain in weight. It appears that this sample of goose fat contained approximately the same amount of vitamin A as the fat from the Plymouth Rock hens (fig. 8). It seems, also, that the fat from the Toulouse geese was much richer in vitamin A than the flesh from the same birds (compare figs. 5 and 10).

VITAMIN A IN TURKEY FAT

In Figure 11 are shown the growth curves of rats fed a ration containing 10 per cent of fat from the White Holland turkey. Growth was considerably below normal. Comparing figures 11 and 6, it will be noted that the flesh from this turkey apparently contained much less vitamin A than did the fat from the same bird. This sample of turkey fat appeared to contain appreciably less vitamin A than the sample of goose fat (fig. 10).

SUMMARY OF RESULTS

In this paper are reported the results of a study of the concentration of vitamin A in the dried flesh from two lots each of chickens, ducks, geese, turkeys, and guinea fowls, and in the fat from two lots of chickens and one lot each of turkeys and geese.

The flesh from one lot each of ducks and of guinea fowls was found to be relatively rich in vitamin A; one sample of chicken flesh contained a fair amount of this vitamin; but the other samples of guinea fowl, duck and chicken flesh, and both samples of goose and turkey flesh, were relatively poor in vitamin A.

One sample of chicken fat and the sample of goose fat each contained a fair amount of vitamin A, but the sample of turkey fat was rather poor in this vitamin.

In each instance it was found that the sample of poultry fat was considerably richer in vitamin A than the corresponding sample of flesh.

On account of the limited number of samples of each kind of poultry flesh and fat which were tested for vitamin A, no general conclusions concerning the value of any one of these products as a source of this vitamin are justified. Additional information concerning the vitamin A content of poultry flesh and fat is to be desired.

BUD VARIATION AND CHIMERAS IN MATTHIOLA INCANA R. BR.[1]

By HOWARD B. FROST

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INTRODUCTION

A mutant form of Matthiola described under the name Slender (*4*)[2] has been determined by Miss Mann (*6*) to be trisomic. Slender plants have 15 somatic chromosomes, while normals have 14. Genetic evidence (*6*) indicates that the extra chromosome of Slender is derived from the pair which carries the genes for single (normal) and double (petalomanous, sterile) flowers.

A case of bud variation has been described by the present writer (*5*),[3] in which part of a plant was Slender single and the rest was normal double. This variation has been ascribed (*6*) to loss of one chromosome in early somatic development, changing a Slender single with one gene for singleness (Ddd) into a normal double (dd).[4] The abnormal intermediate nature of the doubleness was ascribed to the formation of a periclinal single-double chimera as a result of the chromosomal change.

OBSERVATIONAL DATA

The present paper reports three cases of somatic variation and two of intermediate double flowers, all evidently to be explained in the same way as the bud variation previously described. Four of these variant plants occurred within a white, glabrous variety originally obtained under the name Snowflake (*4*), and the other plant came from a cross with a pure single race. Snowflake is a "double-throwing" variety, such as Miss Saunders (*7*) has shown to be permanently heterozygous, the pollen of the singles transmitting only doubleness while their eggs transmit singleness also. The average proportion of the sterile doubles from normal Snowflake parents has been about 53 per cent. To explain the usual excess of doubles over 50 per cent in such cases, Miss Saunders (*7*, *8*) has postulated two complementary dominant genes for singleness, both heterozygous in double-throwers. To explain the absence of singleness from the functional pollen, the writer assumes the presence of a pollen lethal (*3*). Snow (*9*) states that Haldane has devised for the inheritance of doubleness a gamete-lethal scheme which fits the data remarkably well. Whatever is the best complete explanation for the single-double ratio, however, the

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[2] Reference is made by number (italic) to "Literature cited," p. 46.

[3] When the account cited was written, it seemed probable that the Slender type depended on a mutant gene rather than on an extra chromosome. This view was based especially on the fact that two very dissimilar mutant forms, Slender and Crenate, both give apparent linkage ratios for doubleness. Both these forms, however, are trisomic; the somatic differences between them are doubtless related to a difference in the average length of the odd chromosome (*6*). Evidently the odd chromosome of Slender, at least, is a fragment.

[4] Since doubleness obviously is a mutant character, the symbols D and d are used in place of S and s of an earlier paper (*3*). To indicate the pollen lethal about to be mentioned, D′ might be substituted for D in the case of the double-throwers.

simple formulae given above seem adequate for the present purpose. Slender is known to carry an extra chromosome, hence a somatic variation which changes Slender to normal evidently involves the loss of that extra chromosome. If singleness changes to doubleness at the same time, plainly the lost chromosome carried some gene (D) essential to singleness.

The most significant new evidence is given by another change from Slender single to normal double, discovered in February, 1924, among progeny of a selfed Slender parent. In this case there is no room for doubt as to which was the original type of the plant, since the double flowers and normal leaves were confined to one main branch. The double flowers, as in the earlier case, suggested a chimeral condition; usually there were four large petals, within

FIG. 1.—Flowers and leaves from the first plant described in this paper. At the right below, two rosettes with narrow leaves, from the Slender part of the plant; above each rosette, the corresponding raceme, with ordinary single flowers. At the left above, two intermediate or partially double flowers from the variant branch. Second from the left below, a fully double flower from a shoot borne by the variant branch. At the extreme left below, a shoot from the variant branch, with buds apparently fully double and with leaves broader and thicker than those of the Slender shoots

these there were smaller curled petals which sometimes bore traces of anthers, and in some flowers there was an abnormal pistil. One shoot, however, seemed to have ordinary double flowers.

In February, 1925, this plant was again in bloom, but it died before all the new shoots on the variant branch had produced well-developed flowers. All but one main branch was Slender single as before (fig. 1). On the variant branch, one shoot, with several racemes, bore ordinary double flowers, one of which is shown in Figure 1. Another shoot had buds which were probably also fully double. Most (apparently all) of the other five shoots produced abnormal intermediate flowers like those of the preceding season.

In this case and that previously reported, it would be expected that the intermediate flowers would be accompanied by leaves intermediate between Slender and normal, rather than by strictly normal leaves. On account of the great nongenetic variability of the leaves in general, it was not possible to determine this point. There was,

however, a marked change from the slender shoots and narrow leaves of the Slender form, in the direction of the normal type.

Another plant, from a Slender parent, had one vigorous variant branch with normal leaves and ordinary double flowers. The rest of the plant was very small, not as large altogether as the one variant branch; it bore extremely small leaves and single flowers. The single-flowered part in this case may have been Slender, dwarfed by the early development of a branch of the more vigorous normal type, or it may have been a weaker "secondary" trisomic type carrying an extra chromosome different from that of Slender.

In the latter case, presumably, a trisomic single plant produced a normal double by elimination of a chromosome which carried a gene necessary for single flowers. In the former case reported here the process doubtless was the same, except for extensive development of a chimeral condition. If the change in the former case had been due to the production, by gene mutation, of a new type intermediate between ordinary singles and ordinary doubles, the later occurrence of a second mutation changing the new type to ordinary double would have been a remarkable coincidence—unless the intermediate gene was exceptionally unstable. Further, if any of the three bud variations mentioned above arose by gene mutation, the simultaneous disappearance of the trisomic characters would be extremely improbable. Therefore, without having chromosome counts from these plants, one may be very confident that the cytological change consisted in the loss of a chromosome carrying a gene necessary for single flowers.

Another little plant of Slender parentage, itself apparently Small leaved (formerly Small, smooth leaved) (*4*), bore flowers that were all intermediate between singles and normal doubles. It presumably was a chimera, but the fertilized egg which produced it may have carried two extra chromosomes, one of which was later lost.

Still another plant with intermediate flowers throughout came from pollination of pure single by Slender double-thrower. Considered in connection with the first case described in this paper, it is highly probable that this plant was a single-double chimera. Since, however, the leaves appeared strictly normal, while the flowers were far from being fully double, it is possible that the double component of the chimera was produced by gene mutation, or at least in some other way than by nondisjunction of a whole chromosome. If this plant was originally a normal heterozygous single (dD) with 14 chromosomes, and the variant tissue was double (dO) with 13 chromosomes, the loss of a chromosome had surprisingly little effect on leaf form and vigor of growth. A Datura form (*1*) lacking one chromosome in its hypodermal layer differed only slightly from the normal diploid type, but another form, more abnormal, also lacked the same chromosome, and this difference, it is suggested, may have been due to a chimeral condition in the former case.

The flowers of this Matthiola form were highly variable, but, typically, they had two successive sets of floral parts from sepals to stamens (fig. 2). The stamens of the first set were more or less petaloid. Sometimes there was a recognizable pistil at the center of the flower, but often, at least, the axis was further proliferated. During several months of vigorous flowering, no ordinary flowers, either double or single, were observed.

The third new case of obvious somatic variation (if the two plants just described are to be classed as doubtful) occurred in a plant which as a young seedling seemed to belong to the Smooth-leaved trisomic type; this plant came from the parentage Smooth × normal. Smooth is a trisomic form which (fig. 3) is usually smaller and later in flowering than the normal type of the same general genetic constitution. When in bloom this plant appeared normal in vigor, leaf form, and earliness. It was discovered, however, that the plant consisted mainly of one large branch of normal type, while the remainder was unquestionably Smooth. Smooth does not give trisomic ratios for doubleness, and both parts of the variant plant had double flowers. Here again is a somatic variation clearly dependent on elimination of a chromosome.

FIG. 2.—On the left, two racemes from an F_1 plant resulting from pollination of a pure single by a Slender (trisomic) double-thrower, showing normal single flowers and capsules. On the right, two racemes from a sib of the plant just mentioned, showing abnormal double flowers, the lower ones proliferating, as in ordinary doubles, to form branch racemes

DISCUSSION

As the bud-variation type in all these cases had sterile flowers, no genetic test was possible, and counting of somatic chromosomes has not been attempted. In Datura (*1*), however, where trisomic and tetraploid types have been identified as bud variations on normal plants, genetic tests have given positive results, and in another bud-variation form of Datura the loss of one chromosome has been cytologically demonstrated.

The fact that most of the variations mentioned in this paper involve flower form may be due to the definite and conspicuous nature of these flower characters. Changes similar to that in the last case would be much more likely to be overlooked entirely, or to be recorded as doubtful.

It is probable that changes from trisomic to normal in Matthiola are much more likely to become visible than are changes in the

reverse direction, even if the two classes of change occur with equal frequency. When trisomic and normal cells are present in the same plant, the greater vigor of the normal type would doubtless favor its predominance in early stages, as it so strikingly did in later stages in some of the plants described here. In fact, most of the variations described here may have been due to simple somatic nondisjunction of an odd chromosome, producing daughter cells with respective complements of 14 and 16 chromosomes. For, while the vigor of the normal 14-chromosome cell would favor its multiplication, the decided weakness of the tetrasomic type might be expected to lead to its elimination.

These cases add a little to the evidence in favor of Emerson's (*2*) conclusion that recognizable bud variations seem, in general, to be

FIG. 3.—F_1 progeny from a cross between a trisomic form (large-leaved) and a normal pure single. At the left, a Smooth-leaved trisomic mutant, smaller and later than the normal in the middle. At the right, a large-leaved trisomic, also late

more often due to unequal mitosis than to gene mutation. In view of the probability of severe selective elimination of forms with abnormal numbers of chromosomes, it is likely that bud-variation types actually originate far more often in the former way. Gene mutation is, of course, much handicapped in the production of visibly variant types, by the ineffectiveness of recessive mutation in material homozygous for the gene affected. It is possible, however, that somatic elimination of chromosomes is more frequent in trisomic plants than in normals.

Three of the Matthiola bud variations give a little evidence for the chromosome theory of heredity, evidence distinct from that given by the discovery of the association between extra chromosomes and mutant somatic characters. The simultaneous disappearance of singleness and trisomic characters suggests that singleness depended on the chromosome which was lost, and this conclusion is strongly reenforced by the fact that Slender parents give single-double progeny ratios indicating the presence of three allelomorphs (Ddd) for these characters.

Evidence on the nature of bud variation in annual plants is valuable not only as a contribution to general genetic theory, but also for the suggestions it may give as to the probable mode of origin of bud variations in clonal varieties, such as those of Citrus fruits.

SUMMARY

In four cases—three of them described for the first time in this paper—a trisomic, or (in one case) evidently trisomic, type of Matthiola has undergone somatic change to or toward the normal diploid type. In three of these plants the loss of trisomic characters was accompanied by change from single to double flowers. In two plants a periclinal single-double chimera evidently was formed, as was indicated by the intermediate condition of the double flowers and by a further change in one plant to fully double flowers.

Loss of a chromosome evidently was responsible for these bud variations, and probably also for the production of intermediate flowers in two other plants.

LITERATURE CITED

(1) Blakeslee, A. F., and Belling, J.
1924. chromosomal chimeras in the jimson weed. Science (n. s.) 60: 19–20.

(2) Emerson, R. A.
1922. the nature of bud variations as indicated by their mode of inheritance. Amer. Nat. 56: 64–79.

(3) Frost, H. B.
1915. the inheritance of doubleness in matthiola and petunia. i. the hypotheses. Amer. Nat. 49: 623–636, illus.

(4) ———
1919. mutation in matthiola. Calif. Univ. Pubs., Agr. Sci. 2: 81–190, illus.

(5) ———
1921. an apparent case of somatic segregation involving two linked factors. Amer. Nat. 55: 461–464.

(6) ——— and Mann, M. C.
1924. mutant forms of matthiola resulting from non-disjunction. Amer. Nat. 58: 569–572.

(7) Saunders, E. R.
1911. further experiments on the inheritance of "doubleness" and other characters in stocks. Jour. Genetics 1: 303–376, illus.

(8) ———
1916. on selective partial sterility as an explanation of the behavior of the double-throwing stock and the petunia. Amer. Nat. 50: 486–498.

(9) Snow, R.
1924. counted grain pollinations in matthiola. Amer. Nat. 58: 316–321.

THE PEA APHID AS AN ALFALFA PEST IN KANSAS [1]

By ROGER C. SMITH, *Associate Professor of Entomology* and EDGAR W. DAVIS, *Graduate Student, Kansas State Agricultural College*

INTRODUCTION

During the last decade the pea aphid, *Illinoia pisi* (Kalt), has become a major insect pest of alfalfa in Kansas. The first known outbreak occurred during the spring of 1921, and was rather general over Kansas and neighboring States. This aphid has long been known as an enemy of peas and less commonly of red and crimson clovers. Apparently it is extending its range of food plants, and bids fair to continue to be one of our most destructive alfalfa insects. This paper is based on observations of three outbreaks of this aphid, over a period of five years.

Some of the most important writings on the pea aphid are those of Sanderson (*6*)[2], Chittenden (*1*), Folsom (*4, p. 138–154*), Davis (*2*), and Smith (*7*). Published work has dealt with this insect almost exclusively as a pest of peas and clovers. This account is based entirely on rearings and field observations on alfalfa.

OUTBREAKS AND DISTRIBUTION IN KANSAS

The first outbreak in the spring of 1921 followed a mild, dry winter and an early advent of spring (figs. 1 and 2). The temperature at Manhattan was somewhat above normal, while the rainfall was below normal. In the fall of 1920, pea aphids were very abundant in some fields at Manhattan until the alfalfa was killed by frosts early in November. The spring of 1921 set in unusually early. There was a period of typical spring weather in February, and the aphids, already in the alfalfa, multiplied rapidly. New growth of alfalfa appeared rather slowly, as rain was badly needed. By early March the alfalfa over much of the State was heavily infested. Many fields made very little growth. On March 27 there was a very severe freeze, the temperature dropping to 18° F. The new growth of alfalfa was frozen, but the aphids were not perceptibly affected by the freeze. They clustered on the new growth and living parts of the plants in such numbers that it was impossible for the alfalfa to grow. As a result of the infestation and the late freeze, about 100,000 acres of alfalfa in Kansas were killed.[3]

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[1] Received for publication Nov. 19, 1925, issued July, 1926. Contribution No. 347 from the Entomological Laboratory, Kansas State Agricultural College. This paper embodies some of the results obtained in the prosecution of project No. 115 of the Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 57.

[3] Secretary Mohler of the Kansas State Board of Agriculture (*5*), estimated the loss for that year as follows: "The belated first cutting of alfalfa is estimated at 650,000 tons, or slightly less than six-tenths of a ton to the acre. The first cutting last year amounted to 1,271,000 tons, or 1.01 tons to the acre. The damage to alfalfa by spring freezes and attacks of the pea aphis is clearly shown, not only in this exceptionally low yield, but in the impaired vitality of the plant as indicated by its slow recovery through the late maturity of the first cutting. Added to this is the fact revealed by the assessors' returns as of March 1, that the alfalfa acreage has decreased by 128,000 acres, and a further, though as yet undetermined, loss has been sustained since as a result of unseasonable freezing and depredations of insects."

The aphids disappeared rather suddenly. On April 11, 1921, the alfalfa was still heavily infested, but on April 22 only a few aphids could be found. An increasingly large number became alate and flew to other fields, permitting the alfalfa to get a start. The aphids were present in very small numbers in local fields during the remainder of the year. They were locally plentiful, especially on young alfalfa, during the fall of 1921 and the spring of 1922 in several places in the Kaw Valley, though very scarce at Manhattan. The damage, except in a few small areas, was not serious, however, for heavy spring rains brought on a luxuriant growth and at the same time checked the aphids. The aphids were present in local fields, however, until the middle of June and then disappeared. During November they were

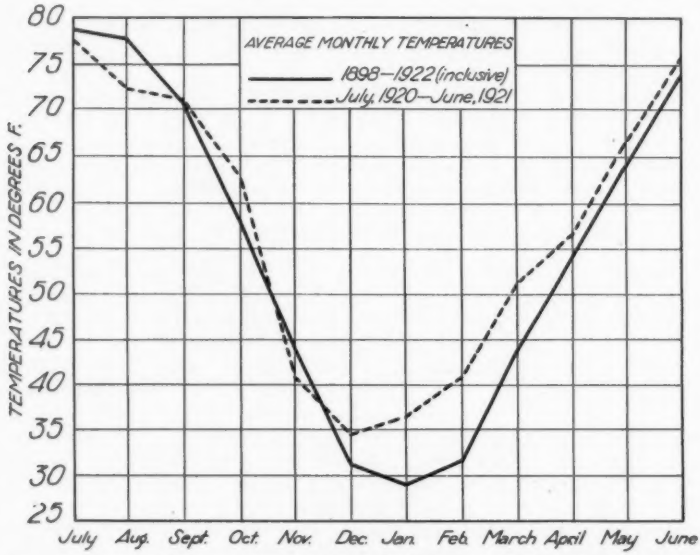

FIG. 1.—Average monthly temperatures for the 25 years, 1898–1922, inclusive, and for the 12-month period from July, 1920, to June, 1921, inclusive, at the Kansas State Agricultural College, Manhattan, Kans.

searched for at Manhattan with especial diligence, but only two were found.

In the spring of 1923, local outbreaks again occurred in the Kaw Valley from Wamego to Kansas City. There were also small, scattered infestations elsewhere in the State. Often only small areas in fields showed injury, but occasionally an entire field was completely killed. Small numbers were observed in fields near Manhattan during the fall of 1923, but they were more abundant in fields between Manhattan and Topeka.

A small and restricted outbreak occurred at Manhattan during April and May, 1924. The insects were present at the same time in small numbers over the larger part of Kansas. Some injury occurred to fields at Lincoln, Kans., and at different points in the Kaw Valley.

A few aphids were observed in alfalfa fields every week of the summer. Small areas were found at Manhattan during the fall of 1924 where they were fairly plentiful, though not doing injury.

SEASONAL HISTORY

The pea aphid is present during the entire year in alfalfa fields. On alfalfa at Manhattan it overwinters chiefly as an apterous viviparous female, and sometimes in the egg stage also. The adults survive best in winter in low areas and more protected parts of fields. Areas protected by hills, buildings, or trees seem to be especially favorable. Heavy growths of alfalfa left to freeze in the fall also seem to be protective.

Though careful searches were made for eggs, none was discovered at Manhattan until the autumn of 1924, when they were found in considerable numbers on the upper and lower surfaces of leaves and on stems of alfalfa plants.

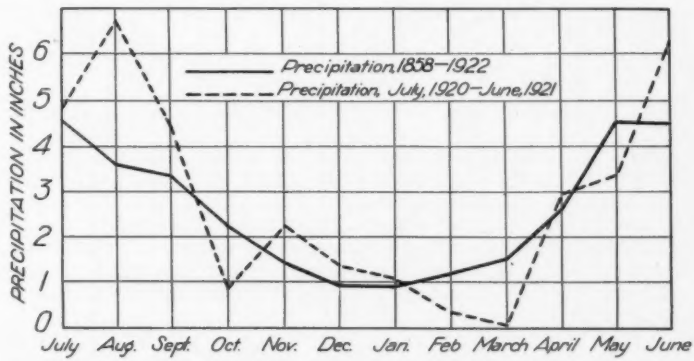

FIG. 2.—Average monthly precipitation for the 65 years, 1858–1922, inclusive, and for the 12-month period from July, 1920, to June, 1921, inclusive, at the Kansas State Agricultural College, Manhattan, Kans.

HOST PLANTS AND INJURY TO THEM

Alfalfa and sweet clovers (Medicago) and white and crimson clover (Trifolium) have been recorded as host plants for pea aphids. The aphids also have been taken repeatedly on vetch at Manhattan. After a spring outbreak they become numerous on both common garden peas and sweet peas. Winged forms have been taken on many other plants, but they were not observed to reproduce upon them.

The injury to alfalfa is distinctive, and a heavily infested spot may be discerned at a considerable distance. The plants are short and bunchy, the tops light green in color, and the lower leaves yellow or dead, the general appearance from a distance being brownish. The bare ground shows up from lack of covering, and it is usually heavily besprinkled with the whitish aphid skins or molts. The aphids which cluster on both the stems and under side of the leaves show a preference for the younger growth. If the infestation continues, the leaves wither and the plants eventually die. The aphids leave, how-

ever, before the plants are dead. These aphids have been observed feeding or attempting to feed on wild mustard, shepherd's purse, and other weeds, when driven to it by the death of alfalfa. The alfalfa acreage in Kansas has decreased from 1,360,000 acres in 1915 to 884,000 acres in 1924, and the pea aphid has been an important factor in this decrease.

WINGED AND WINGLESS FORMS

The percentage of winged forms gradually increases as spring advances. Observations indicate that as they become crowded on the alfalfa plants, or that as the plants begin to weaken an increasingly large number of progeny is winged, thus enabling them to scatter. However, this condition may be partly cyclical, also.

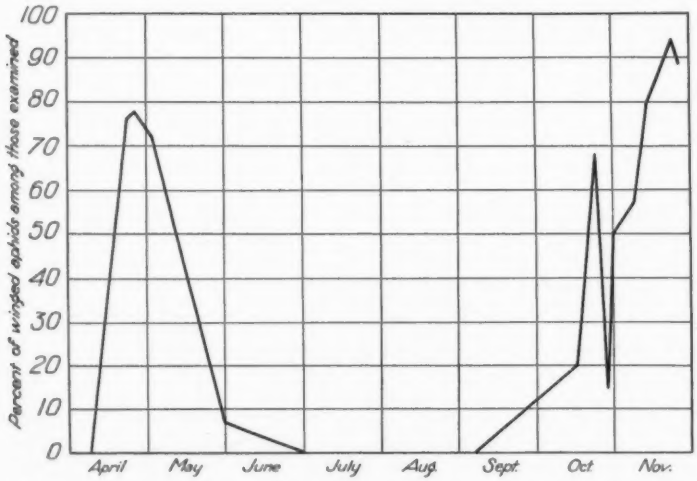

FIG. 3.—Percentage of aphids that were winged among those that were examined in various months of 1924

Field collections of pea aphids were made every week during the summer of 1924, beginning April 8. The number taken varied because of scarcity in the fields, but whenever possible, 300 was the number counted. Only winged adults or apterous adults in which the eye spots of their young could be seen in their abdomens were counted. The results of these counts for one year are indicated in Figure 3. This graph shows that in the early spring this aphid was predominately wingless. Some time between April 8 and 24 the winged forms equaled and on April 24 exceeded the wingless forms. On May 5 the winged forms reached their maximum and then decreased. Only wingless forms were found during the summer. In the fall the winged forms increased in an irregular manner until in November they again predominated. There were not sufficient aphids during the spring and summer of 1925 to repeat these counts.

CLIMATIC INFLUENCES

The numbers and activity of the pea aphid in the field are controlled very largely by climatic conditions. The outbreaks thus far observed indicate that this insect thrives best when the winter is mild and relatively dry. Early rapid growth of alfalfa, especially in March and April, greatly reduces or eliminates the possibility of serious damage by this insect. The three outbreaks followed a warmer and drier February than is the average, and March had a somewhat reduced precipitation and was warmer than the average. The aphids reproduce slowly at a lower temperature than that in which lady-bird beetles become active. Temperatures of 90° to 100° F. are clearly unfavorable, especially when accompanied by low humidity. A temperature of 100° or higher was usually quickly fatal in the temperature-control cages. This agrees in the main with the conclusions of Smith (7), though the temperature and humidity optimum of Virginia is apparently somewhat higher than in Kansas.

The well-known fungous disease which develops under humid conditions and which has been reported frequently as being the most important natural control of this aphid, has been less important during these outbreaks than predacious insects, especially coccinellids.

REARINGS IN INSECTARY AND FIELD

Studies on the general biology of the pea aphid were begun in October, 1923, and continued, as material and temperature permitted, until the fall of 1924. The usual lamp-chimney cage over young growing alfalfa plants was used. One series (Y) was reared under temperature-controlled condition and another series (Z) was reared under ordinary greenhouse conditions. The rearings were transferred to a small field laboratory in April, 1924, where the work was carried on during the summer. These results are indicated here as the F series.

Each of the series was started by bringing in from the field a mature female and placing her on alfalfa. Her progeny was transferred to other alfalfa plants as produced, and were numbered Y2 or Z2, etc. A summary of the rearings under the uncontrolled-temperature or ordinary greenhouse conditions is given in Table 1.

In the fall of 1924, rearings were conducted in the greenhouse in the same manner as those shown in Table 1, as a check on the work of 1923. The numbers of young and length of reproductive period of the aphids in this series were slightly greater than for those recorded in Table 1. This may have been due to two conditions: (1) In 1923 the weather was too severe for the aphids to reproduce in the field during the time the experiment was being carried on in the greenhouse, while in 1924 the aphids were reproducing in the field the entire time the experiment was being carried on in the greenhouse, and (2) the technique had been improved, so that it may have been that better care was given to those of the 1924 series.

The averages for the 116 individuals studied in both series in the two years are as follows: The average age of female when first young was born, 9.7 days; the youngest age, 6 days. The average length of reproductive period was 12.8 days; the longest, 31 days.

The average number of young produced was 39.3; the largest number, 93. The average length of life was 25.1 days; the longest life, 43 days.

TABLE 1.—*Studies of life histories of pea aphids under greenhouse conditions, 1923–24*

Series Z								Temperature			
Generation	Date of birth	Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Maximum	Minimum	Average hourly	Humidity, average
								°F.	°F.	°F.	
1[a]											
2	Nov. 9	7	6	23	5	Nov. 22	13	97	65	77	75
3	Nov. 16	13	4	15	5	Dec. 3	17	100	63	77	73
4	Nov. 22	8	2	12	11	Dec. 2	10	100	63	77	73
5	Dec. 1	7	5	14	5	Dec. 13	12	97	65	76	78
6	Dec. 9	11	8	20	4	Dec. 28	19	100	70	79	77
7	Dec. 20	8	6	20	4	Jan. 3	14	96	70	78	80
8	Dec. 27	8	6	18	9	Jan. 11	15	95	60	76	80
9	Jan. 4	10	5	19	8	Jan. 19	15	97	60	77	79
10	Jan. 13	8	3	11	6	Jan. 24	11	97	65	70	79
11	Jan. 21	11	18	47	8	Feb. 20	30	97	60	79	80
12	Feb. 1	7	12	53	11	Feb. 20	19	95	61	79	78
13	Feb. 9	7	31	58	6	Mar. 25	45	97	61	78	80
14	Feb. 17	8	16	84	14	...do...	37	97	61	78	80
15	Feb. 25	8	20	69	13	Mar. 31	35	98	61	77	76
16	Mar. 5	6	13	78	14	Apr. 7	33	98	60	76	75
17	Mar. 11	9	18	77	14	Apr. 8	28	100	62	76	77
18	Mar. 20	7	16	93	14	Apr. 12	23	100	60	76	80
19	Mar. 27	9	8	44	7	Apr. 14	18	100	60	77	81
20	Apr. 5	7	5	24	11	Apr. 18	13	100	60	79	80
21	Apr. 12	----	(b)								
		159	202	779			407				
Average of 19 individuals		8.3	10.6	41			21.4				

[a] Caught wild, record incomplete.

[b] Died.

Folsom (*4*, *p. 138–154*), recording the work of Webster, gives the following averages for 16 life histories reared in a line on peas from March to September: The average number of days to maturity, or the beginning of reproduction, was 11; length of life, 25.4 days; average number of young, 46. Davis (*2*) obtained the following averages: Age at which females reproduce "varied from 6 to 35 days, with an average of 10.3 days, from 38 records covering the months from May 15 to December." Average of all rearings over two years was 12.1+ days; length of reproductive period varied from 2 to 68 days, or an average for 53 examples over two years of 22 days; total length of life, 10 to 85 days, or an average of 39.1+ days over two years; average total number of young produced was 68.3, with one instance of 124 as a maximum.

The results of the three series of rearings reported in this paper differ from the above results chiefly in that the Kansas aphids began to reproduce after a slightly shorter post-natal period and their reproductive period and their average length of life were both somewhat shorter also.

The experiments the results of which are recorded in Table 2 were carried on in one of the temperature-controlled chambers described by Dean and Nabours (*3*). The temperature shows a

rather wide range from maximum to minimum, but these extremes were in the form of peaks and remained at these points only a few minutes. The averages of the different phases of the life cycle recorded here show a decided increase over those recorded in the preceding table. The number of days of the reproductive period in the constant-temperature cages was 16.4 days, compared to 10.6 days in the greenhouse. The average number of young in this experiment was 54.3 in contrast with the 41.0 in Table 1. The length of life was also longer in the controlled temperature. The largest number of young from one female was 121 (Table 2), which occurred during the time when the average temperature was 65° F. The temperature in the experiment at the start was above 70°, and continued to be that for about a month. When the average temperature was decreased to 65° there was a decided increase in the number of young produced. A decrease in the number of young per individual occurred when the temperature was again raised to 70°. This decrease, however, was not as marked as the increase resulting from lowering the temperature from 70° to 65°. The differences may have been due to the conditions under which the aphids were kept during nymphal development. It seems probable that the optimum temperature for this aphid is between 60° and 70°, though no definite conclusion can be drawn, for it was impossible to hold the temperature for any length of time at points below 60°. Hence, no data on lower temperatures are available.

TABLE 2.—*Life histories of pea aphids under controlled temperature conditions, 1924*

Series Y		Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Temperature			Humidity, average
Identification No.	Date of birth							Maximum	Minimum	Hourly average	
								°F.	°F.	°F.	
8 (1)	Jan. 4	9	2	12	6	Jan. 15	11	83	70	75	74
9 (3)	Jan. 13	7	8	39	7	Jan. 28	15	80	60	70	75
10 (1)	Jan. 20	10	19	19	7	Feb. 17	28	80	60	70	78
11 (1)	Jan. 30	11	8	17	3	Feb. 17	19	80	60	70	78
12 (1)	Feb. 10	11	24	75	7	Mar. 21	40	80	57	65	78
12 (2)	Feb. 10	10	19	98	10	Mar. 20	39	80	57	65	78
13 (2)	Feb. 20	10	18	121	11	Mar. 29	38	80	55	65	78
14 (1)	Feb. 29	9	30	87	12	Apr. 8	39	90	57	70	81
14 (2)	Feb. 29	11	20	68	7	Apr. 8	39	90	57	70	81
15 (1)	Mar. 9	9	21	90	12	Apr. 8	30	87	55	70	81
15 (2)	Mar. 9	10	19	97	9	Apr. 8	30	87	55	70	81
16 (1)	Mar. 19	8	12	64	14	Apr. 8	20	85	55	70	70
16 (2)	Mar. 19	8	10	65	10	Apr. 8	20	85	55	70	80
17 (1)	Mar. 28	8	7	35	9	Apr. 16	19	85	55	70	81
10 (2)	Jan. 13	8	5	14	5	Jan. 26	13	83	70	70	76
11 (2)	Jan. 21	8	16	29	7	Feb. 28	38	80	60	70	78
11 (3)	Jan. 27	12	19	22	4	Feb. 27	31	80	60	70	78
11 (4)	Jan. 29	12	17	20	3	Mar. 5	35	80	57	70	81
11 (5)	Jan. 29	12	7	12	4	Mar. 13	43	80	57	70	81
12 (1)	Jan. 29	10	7	17	5	Feb. 15	17	80	60	70	81
12 (2)	Jan. 29	10	32	55	9	Mar. 10	42	80	55	65	80
13 (1)	Feb. 8	9	13	51	6	Mar. 3	24	80	57	70	80
13 (2)	Feb. 8	10	32	67	5	Mar. 24	45	80	57	65	81
14 (1)	Feb. 17	11	26	111	10	Mar. 25	37	80	55	65	80
14 (2)	Feb. 18	10	26	95	7	Apr. 8	50	90	55	70	80
14 (3)	Feb. 17	12	28	75	7	Apr. 7	50	90	57	70	81
14 (4)	Feb. 18	11	11	54	9	Mar. 13	24	80	55	70	80
15 (2)	Feb. 28	9	8	30	6	Mar. 6	18	90	57	70	80
16 (2)	Mar. 9	8	12	53	9	Mar. 29	20	87	55	70	80
17 (1)	Mar. 18	8	16	37	6	Apr. 15	28	85	55	70	80
Av. of 30 individuals		9.7	16.4	54.3			30				

The experiments the results of which are recorded in Table 3 were carried on in the field laboratory during the summer of 1924. It was difficult to rear the aphids over a complete life cycle. However, the aphids were also decreasing in numbers in an adjoining alfalfa field, so the insectary equipment apparently was not responsible for the difficulties encountered in the rearing in the insectary. Attempts during the summer of 1925 to rear an unbroken line through the summer on alfalfa at the field insectary were unsuccessful.

A comparison of the data obtained under field-insectary and greenhouse conditions is of interest. The average age of the female when first young was born in the field insectary was 10.6 days, and in the greenhouse it was 8.3 days. The average length of the reproductive period in the field insectary was 3.3 days, and in the greenhouse it was 10.8 days. The average number of young produced in the field insectary was 13.4 and in the greenhouse 21.4. In the field insectary, as well as in the greenhouse, the largest number of young was produced when the average temperature was about 60 or 65° F. Also, the aphids lived longest at these temperatures. However, under both of these conditions the averages were not nearly so high as when the temperature was more constant. In the greenhouse, where the variation in temperature was greater than that in the air chambers, the averages of the life activities of the aphids were smaller.

TABLE 3.—*Life histories of pea aphids under field-insectary conditions, 1924*

Series F		Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Temperature			Humidity, approximate average
Identification No.	Date of birth							Maximum	Minimum	Average	
2 (8)	June 30	10	2	6	3	July 12	12	98	46	74	72
2 (1)	May 1	20	1	2	2	June 1	31	90	35	60	71
3 (1)	May 19	12	10	66	7	June 13	25	95	35	65	81
3 (2)	May 19	12	7	42	10	June 9	21	95	35	62	80
4 (1)	May 30	9	8	23	8	June 16	17	95	47	75	79
5 (1)	June 8	8	5	13	6	June 23	15	104	60	80	79
6 (2)	June 16	10	2	3	2	June 30	14	104	58	80	80
7 (1)	June 26	12	1	2	2	July 9	13	100	46	75	75
2 (1)	May 5	18	1	9	9	June 1	27	90	35	60	70
3 (1)	May 23	12	3	19	7	June 9	17	95	35	65	70
3 (2)	May 23	12	2	9	5	June 9	17	95	35	65	70
4 (1)	June 4	8	3	11	7	June 17	13	95	47	75	80
4 (2)	June 8	7	3	12	5	June 19	11	104	60	80	80
2 (1)	June 24	12	8	20	5	July 15	21	100	46	75	80
2 (2)	June 24	10	2	8	4	July 8	14	100	46	75	75
2 (3)	June 24	10	3	11	4	July 9	15	100	46	75	75
3 (6)	July 7	8	1	10	10	July 17	10	104	55	80	70
3 (8)	July 7	8	1	8	8	July 17	10	104	55	80	70
2 (1)	June 30	10	6	15	4	July 17	17	104	55	77	73
2 (2)	June 30	11	1	3	3	July 12	12	97	55	75	73
2 (3)	June 30	11	2	4	2	July 13	13	97	55	75	73
2 (5)	June 30	10	3	10	8	July 13	13	97	55	75	73
2 (14)	July 2	10	1	3	3	July 14	12	97	55	77	70
Av. of 23 individuals		10.8	3.3	13.4			16				

CORRELATION RATIOS

Correlation ratios and probable errors were computed for several factors of the life cycle of the pea aphid. The averages from Tables 1 and 2 were used. The formula used for the correlation computation was:

$$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{N}}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right]\left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right]}}$$

X and Y are the original numbers, and N is the total number of individuals. The probable error was computed by the following formula, r being the correlation ratio:

$$\pm \frac{0.6745\ (1-r^2)}{\sqrt{N}}$$

When the correlation is 4.9 times the probable error, the results are regarded as significant; that is, according to the laws of chance, such condition would happen only once in 1,052 trials.

These determinations were made to show the presence or absence of correlations, not only between the different factors of the life cycle of the pea aphid, but also between these factors and the average hourly temperature. These determinations are given in Table 4.

TABLE 4.—*Correlation between various factors in the life cycle of pea aphids*

Between	Data from Table 1		Data from Table 2	
	Correlation coefficient	Probable error	Correlation coefficient	Probable error
Age of mother when first young was born and—				
Length of reproductive period	−0.1695	±0.1503	0.3522	±0.1079
Total number of young	−.3042	±.1404	.06081	±.1226
Length of life	−.1006	±.1532	.5583	±.0848
Average hourly temperature	.1854	±.1494	−.227	±.1168
Length of reproductive period and—				
Total number of young	.7588	±.06559	.6209	±.0756
Length of life	.9324	±.02033	.8242	±.0395
Average hourly temperature	−.01248	±.1547	−.5971	±.07926
Total number of young and average hourly temperature	−.2288	±.1466	−.5624	±.08426
Length of life and average hourly temperature	−.0092	±.1547	−.5277	±.08822

The significant correlations are (1) between the age of the mother when the first young was born and the length of life under controlled conditions; (2) between the length of the reproductive period and the total number of young produced; (3) between the length of reproductive period and the length of life; (4) between the length of the reproductive period and the average hourly temperature in the controlled temperature series; (5) between the total number of young and the average hourly temperature in the controlled temperature series; and (6) between the length of life and the average hourly temperature in the controlled temperature series.

The first three correlations will not be discussed here, for the reason that they are either readily explainable or not important in this discussion. In the latter three correlations, it seems clear that the length of the reproductive period, the total number of young produced, and the length of life of the mother are strongly affected by temperature. It should be remembered that food conditions were kept at or near the optimum, and that the variations in humidity were slight. The data indicate that as the temperature varies from the indicated optimum, the length of life, the length of reproductive period, and the total number of young produced are reduced.

The average length of life under uncontrolled temperatures was 21 days, and the number of young produced was 41, while corresponding data under controlled temperature was 30 days and 54 young.

The largest number of young was produced at an average temperature of 65° F. and a humidity of 80°. As the average temperature increased, the number of young produced decreased. This is especially important in explaining the seasonal activity of these aphids in the field. The aphids are most abundant and injurious in the field in April and May. The 37-year average temperatures for these months for the State are 54.6° and 63.6° F., respectively, which are not far from the indicated optimum.

The aphids become very scarce in early June, and remain so until September. Rearings in the field insectary, as recorded in Table 3, emphasize the reduced rate of reproduction and shortened life, which, with the activities of natural enemies and the cutting of the crop, explain their scarcity in the field. It was almost impossible to keep an unbroken series going in the field insectary during the summer, under the most favorable food conditions, and where natural enemies and the cutting of the alfalfa were not influencing factors. This would indicate that the high summer temperature and low humidity were dominant influences.

In September and October the average temperature approaches the optimum for this insect, and the aphids again become fairly plentiful though not particularly injurious. The average temperatures for these two months for 1920, when aphids were unusually plentiful in certain fields at Manhattan, were 69.6° and 60.8° F., respectively.

SUMMARY

During these studies, three outbreaks of the pea aphid—one of major importance, and the other two of minor importance—occurred in alfalfa fields in Kansas. Observations were made upon the aphid's behavior in the field and upon some features of its life economy, by rearings on alfalfa in a field laboratory, in the chambers of a controlled-temperature apparatus, and in an ordinary greenhouse. The following are the results of the three series of rearings:

Series F: Twenty-three individuals reared on alfalfa in field laboratory; average temperature from May 1, 1924, to July 14, 1924, 73° F.—range 35° to 104°.

Series Y: Thirty individuals reared on alfalfa under controlled-temperature conditions; average temperature from January 4, 1924, to April 15, 1924, was 69° F.—range 55° to 90°.

Series Z: Twenty-one generations reared on alfalfa in greenhouse, under natural greenhouse conditions; average temperature from November 9, 1923, to April 18, 1924, was 77.4° F.—range from 60° to 100°.

TABLE 5.—*Summary table*

	Series F			Series Y			Series Z		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
Age of female when first young was produced (days)	10.8	20	7	9.7	12	7	8.3	13	6
Length of reproductive period (days)	3.3	10	1	16.4	32	2	10.8	31	3
Number of young	13.7	66	2	54.3	121	12	41	93	11
Average number of young per day of reproductive period	3.9	10	1	3.3	6	1	3.7	6	2
Length of life (days)	16.0	31	10	30.0	50	11	21.4	45	10

The optimum temperature for this aphid seemed to be at or near 65° F., at a humidity of about 80 per cent, but the exact optimum could not be determined. The aphids were most plentiful in the field during the months which have an average temperature near 65°—viz, April, May, September, and October. There is a strong indication that the rainfall of March is a governing factor in Kansas, with respect to possible damage to alfalfa. It was difficult, and in some cases impossible, to maintain an unbroken line in the field insectary during the summer. The aphids are very scarce in alfalfa fields also at this time. There is an indication that the high summer temperatures and low humidity are predominating influences, although natural enemies are also a factor. The pea aphids overwinter at Manhattan chiefly as nymphs and adults, but eggs were taken during the winter of 1924.

LITERATURE CITED

(1) CHITTENDEN, F. H.
1901. THE DESTRUCTIVE GREEN PEA LOUSE. U. S. Dept. Agr., Div. Ent. Circ. (2) 43, 8 p., illus.

(2) DAVIS, J. J.
1915. THE PEA APHIS WITH RELATION TO FORAGE CROPS. U. S. Dept. Agr. Bul. 276, 67 p., illus.

(3) DEAN, G. A., and NABOURS, R. K.
1915. A NEW AIR CONDITIONING APPARATUS. Jour. Econ. Ent. 8: 107–111, illus.

(4) FOLSOM, J. W.
1909. THE INSECT PESTS OF CLOVER AND ALFALFA. Ill. Agr. Expt. Sta. Bul. 134: 113–197, illus.

(5) MOHLER, J. C.
1921. [CROP REPORT.] Kans. State Bd. Agr. [Press Bul. June 24, 1921.] 3 p.

(6) SANDERSON, E. D.
1900. THE DESTRUCTIVE PEA LOUSE IN DELAWARE. Del. Agr. Expt. Sta. Bul. 49: 14–24, illus.

(7) SMITH, L. B.
1916. THE GREEN PEA APHID. Va. State Ent. and Plant Path. Rpt. (1914/15) 10: 32–63, illus.

CLIMATIC EFFECTS IN THE METABOLISM OF THE SUGAR BEET[1]

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INTRODUCTION

In the summer of 1919 the senior writers undertook an investigation of nitrogenous metabolism in leaves of the sugar beet, which has been reported briefly (*17, p. 17*).[3] It was the purpose of that work to determine whether protein synthesis occurred simultaneously with carbon assimilation or ensued in the following period of darkness. The results of earlier investigation on this point indicate the first of these relations. Kosutany (*11*) found a little greater percentage of soluble protein in leaves of *Vitis riparia* at 3 a. m. than at 3 p. m. On the other hand, Suzuki (*22*), employing leaves of several plant species, found at 6 p. m. a decided excess of this constituent over the percentage at 6 a. m. Schulze and Schütz (*18*), basing results upon both the number of leaves sampled and the weight of fresh material, concluded that with younger leaves of boxelder (*Acer negundo*) proteins migrated at night from the seat of synthesis. Similarly, Pigorini (*16*) found a nocturnal decline of more than 11 per cent in the protein nitrogen of leaves of mulberry (*Morus alba*). These results were based on weights of fresh tissue. According to Chibnall (*7*), this is the most accurate method of expressing the results. He found (*8*) with the runner bean (*Phaseolus vulgaris*) a decline of nearly 2 per cent of foliar protein nitrogen at night.

ANALYTICAL PROCEDURE

Inasmuch as preliminary tests (*24*) had indicated the impossibility of preserving the tissue without alteration of the soluble protein, the freshly sampled material was extracted directly. The leaf surface was dried by absorbent paper and brushed free of foreign material. After dissecting out the midribs the blade tissue was rapidly chopped fine, thoroughly mixed and replicate samples of 20 or 25 gm. were taken for moisture determination. Replicate samples of 10 gm. were used for determining total nitrogen. A 100-gm. portion of the tissue was triturated rapidly in a large porcelain mortar with the addition of an equal weight of washed, fine, spherical sand. About 5 c. c. of ether was added to promote plasmolysis, and water was added as required to give proper consistency for trituration. This took about 15 minutes. The pulverized tissue was then transferred

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[3] Reference is made by number (italic) to "Literature cited," p. 75.

to a double layer of cheesecloth and extracted with successive portions of water to a volume of about 1,800 c. c., wringing out each portion of wash water. This extract was passed through a layer of paper pulp nearly 0.5 cm. thick on a Buchner funnel, with the aid of gentle suction. Petioles and roots were similarly treated. When washed to a volume of 2 liters, the filtrate was usually quite transparent and green to brown in color. Aliquots of 500 c. c. were brought quickly to boiling and coagulated with a few drops of 10 per cent acetic acid. This precipitate of the soluble protein was filtered out and determined by the Kjeldahl-Gunning method, the time required from sampling of the tissue to boiling of the extract being about one hour. This period is of importance in relation to a subsequent discussion of enzymatic action. The extent of extraction specified here had been found to remove all but traces of soluble aplha-amino nitrogen compounds.

The residual solution from the separation of soluble protein was employed for determination of sugars and of nitrogen present as amino acids and other forms. Separate aliquots from the 2 liters of filtered extracts served for the determination of total soluble nitrogen. Reducing sugars were determined by the Shaffer-Hartmann method (*19*), and sucrose by the Herzfeld process for inversion (*5, p. 266*).

It appears that the amount of nitrogen, apparently in the form of soluble protein, may depend upon the degree of grinding of the tissue, as well as upon the methods of filtering. The writers have found that this constituent is relatively independent of the thickness of paper pulp employed as a filter. Comparison of the composition of extract with that obtained by use of a mill for grinding, as employed by Osborne (*14*) with frozen tissue, is given in Table 1. The writers applied the mill to the fresh, unfrozen tissue.

Microscopic examination indicated that the walls of practically all cells were ruptured by the use of a mortar for grinding. The results show that the mill treatment recovered 10 to 15 per cent more of the total nitrogen in soluble form than did use of the mortar. This is accounted for largely by the increased dispersion of protein. That a similar effect can be obtained by regrinding the extracted tissue in the mortar method is indicated by preliminary tests. The other differences in composition of extract by the two methods of grinding are too small to appear significant.

TABLE 1.—*Composition of extract obtained from sugar-mangold leaves by mortar and pestle, compared with use of the Nixtamal mill*

[Values are percentages of dry matter]

	Sample A		Sample B	
	Mortar	Mill	Mortar	Mill
Total nitrogen	5.40	5.40	5.42	5.42
Soluble nitrogen	3.62	4.20	3.42	4.28
Coagulable nitrogen	2.70	3.28	2.40	3.16
Other soluble nitrogen	.92	.92	1.02	1.12
Alpha amino nitrogen	.35	.22	.31	.22
Reducing sugars	3.00	3.05	2.45	2.60
Sucrose	1.40	1.35	1.60	1.35

A phase of this process of recovering the soluble constituents which should receive special attention is the possibility of inversion of sucrose during the period between sampling and heating the extract for recovery of soluble proteins. To test this matter, extracts obtained by maceration in a mortar, in which the time elapsed until inactivation of enzymes by boiling did not exceed 30 minutes, were compared with those obtained from corresponding leaves killed immediately after sampling by immersion in boiling alcohol (*13*). $CaCO_3$ was added promptly to the extracts. The data appear in Table 2.

TABLE 2.—*Comparison of recovery of sugars from sugar-beet leaves by direct extraction with alcohol and delayed extraction with water*

[Values are percentages of dry matter]

	Time of sampling					
	Morning		Noon		Night	
	Direct	Macerated	Direct	Macerated	Direct	Macerated
Reducing sugars	2.85	2.90	4.74	4.83	1.40	1.49
Sucrose	1.70	1.71	1.35	1.43	2.00	2.10

These results show that no inversion occurred during the time required by the extraction method employed in the present investigation. Similar conditions were found to hold for petiole and root tissues. Preliminary trials over longer periods indicated that inversion of sucrose in leaf tissue does not become appreciable at room temperatures until after one hour. This gives good grounds for assuming that marked variations in the distribution of sugars in the present case existed in the tissues as sampled.

RESULTS OF 1919

Samplings from many plants were taken at about sunrise and sunset. Only two leaves were taken from a single plant, and these were intermediate in age to the foliage as a whole. The climatic data are those recorded at the weather station, located about 1.2 kilometers east of the beet field and at some 30 meters greater elevation than the beet field.

In Table 3 are assembled the climatic and analytical data of this period. A value of 20 per cent has been assumed to approximate the average nitrogen content of soluble compounds other than proteins, amino acids, and ammonia, reported here as "rest-soluble N." All of the values for nitrogenous compounds were based on the dry matter. In this particular case the values for sugar were obtained by the Defren-O'Sullivan method. Therefore they probably exceed the true reducing sugars, and are of questionable significance. The climatic and analytical data are assembled in Figure 1.

It appears from the graphs that the time of day was less significant than temperature in determining differences of composition. The limited and questionable data for reducing sugars seem to be directly related to temperature variations. The soluble protein varies in an inverse manner to changes of temperature, and with particularly

marked effect on the cold morning of August 27. This is true also of the other higher forms of soluble, assimilated nitrogen designated as "rest-soluble." The insoluble protein varies in the same directions as temperature, while α-amino nitrogen varies with temperature in the afternoon samplings, but inversely to it in the morning. Ammonia varies in the manner of temperature throughout, but amide follows the course of amino acid. For some reason not apparent, the data for the evening of September 6 are erratic throughout as compared with other dates. It may be noted that a medium degree of radiation coincides with a high temperature efficiency on this date.

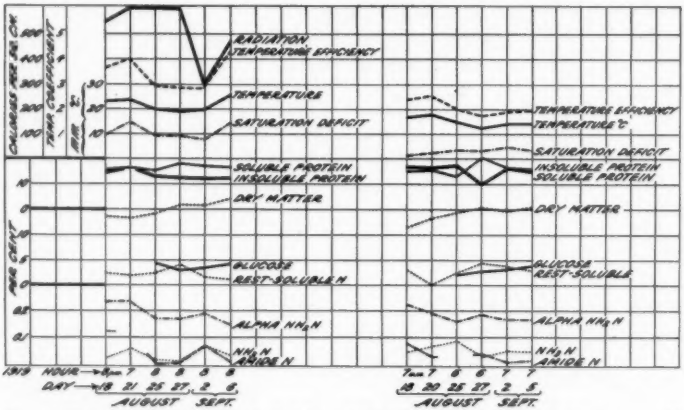

FIG. 1.—Graphs of climatic and analytical data of 1919 investigations of the leaf of sugar mangold

TABLE 3.—*Climatic data and analytical results for investigation of sugar-beet leaves, 1919*

Date	Climatic data				Dry matter	Analytical data of dry matter						
	Temperature	Temperature coefficient	Radiation calories per square centimeter	Vapor pressure deficit		Reducing sugars	Insoluble protein	Soluble protein	Rest-soluble N×5	α-Amino N	Amide N	Ammonia N
	°C.			Mm.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Aug. 18, 7 a. m.	17.5	2.5	.....	1.9	11.9	.....	16.9	14.6	2.9	0.22	0.08	0.05
8 p. m.	23.5	3.7	556	9.5	13.7	.....	16.6	16.9	2.3	.24	.12	.03
Aug. 20, 7 a. m.	17.8	2.5	.....	2.3	13.4	.....	16.7	15.2	.2	.19	.03	.07
Aug. 21, 7 p. m.	24.4	4.0	597	14.3	13.6	.....	17.3	17.4	2.1	.25	.....	.06
Aug. 25, 6 a. m.	14.4	2.0	.....	4.0	14.6	2.5	17.0	12.5	2.6	.16	.....	.08
8 p. m.	20.0	2.9	592	9.4	14.6	4.1	13.5	15.6	2.5	.17	.01	.02
Aug. 27, 6 a. m.	12.8	1.8	.....	3.1	15.2	3.3	9.8	20.5	4.8	.19	.04	.03
8 p. m.	19.4	2.8	585	9.2	16.1	3.2	12.0	18.5	4.1	.17	.02	.02
Sept. 2, 7 a. m.	13.9	1.9	.....	5.2	14.9	3.2	15.5	16.3	3.3	.17	.02	.05
8 p. m.	20.0	2.9	284	7.8	16.1	3.4	11.8	17.9	1.5	.19	.06	.06
Sept. 5, 7 a. m.	14.4	2.0	.....	2.8	15.3	3.6	15.2	15.0	3.2	.17	.02	.05
Sept. 6, 8 p. m.	20.1	4.5	480	14.1	17.3	3.9	12.4	16.3	1.0	.15	.02	.03

RESULTS OF 1920

The dates of sampling in this season were necessarily widely separated. This should be kept in mind in considering irregularities of the analytical data, particularly as regards the widely separated final date of September 29. The data are given in Table 4, and are shown graphically in Figure 2.

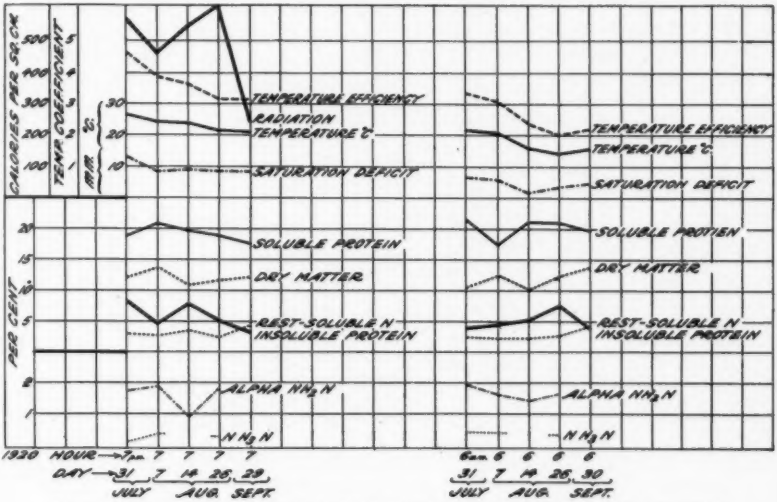

FIG. 2.—Graphs of climatic and analytical data of 1920 investigations of the leaf of sugar mangold

TABLE 4.—*Climatic data and analytical results for investigation of sugar-beet leaves, 1920*

Date	Climatic data				Dry matter	Analytical data of dry matter				
	Temperature	Temperature coefficient	Radiation calories per square centimeter	Vapor pressure deficit		Insoluble protein	Soluble protein	Rest-soluble N×5	α-Amino N	Ammonia N
	°C			*Mm.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
July 31, 6 a. m.	21.7	3.30	.....	6.9	11.0	4.3	21.9	2.6	0.20	0.05
7 p. m.	26.7	4.67	570	12.2	12.5	8.1	18.8	3.1	.17	.02
Aug. 7, 6 a. m.	21.1	3.17	.....	6.3	12.8	4.8	17.5	2.2	.16	.04
7 p. m.	23.9	3.85	459	8.4	12.8	4.6	21.3	2.7	.18	.04
Aug. 14, 6 a. m.	16.7	2.33	.....	1.7	10.0	5.2	21.3	2.2	.14	.06
7 p. m.	23.3	3.70	535	9.6	11.3	7.5	19.4	3.6	.09	.....
Aug. 26, 6 a. m.	14.4	2.00	.....	3.4	12.5	7.5	21.3	2.5	.17	.03
7 p. m.	21.1	3.17	590	8.9	13.2	5.0	18.1	2.5	.18	.03
Sept. 30, 6 a. m.	16.1	2.25	.....	5.2	14.8	3.8	19.4	4.5	.....	.....
Sept. 29, 7 p. m.	21.1	3.17	254	8.5	14.5	3.1	17.5	4.5	.....	.....

In general, soluble protein varied in a manner inverse to temperature changes. This agrees with the results of 1919. Insoluble protein varied somewhat in the same direction as temperature in the evening samplings, but inversely so in the morning. The rest-soluble nitrogen varied irregularly, and apparently insignificantly. α-Amino nitrogen varied in a manner diametrically opposite to its behavior in 1919—that is, in the opposite direction to temperature in the afternoon but directly so in the morning. The data for NH_3 are too few to be significant. It is apparent that the scattered dates of sampling failed to produce consistent analytical results. The marked increase of rest-soluble nitrogen with advance of the season (September 29 to 30) is striking, and simulative of the low-temperature effect on proteins in 1919.

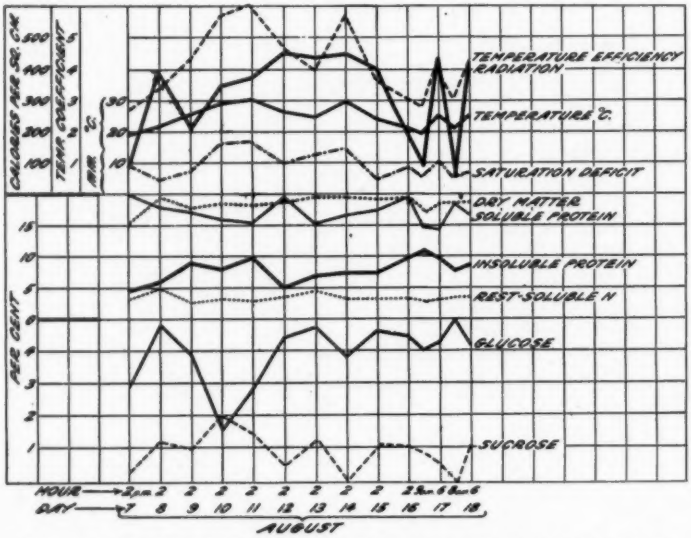

FIG. 3.—Graphs of climatic and analytical data of 1923 investigations of the leaf of sugar beet.

RESULTS OF 1923

In 1923 the services of several workers were available. Even with this unusual amount of assistance it was necessary to abandon detailed separation of the nitrogenous constituents in order to maintain a schedule of two or more samplings per day. More importance was attached, however, to the uninterrupted daily samplings and analyses of petioles and roots, in addition to leaf blades. It appears, also, that it was possible to select more favorable hours of the day than in previous seasons for obtaining marked and significant differences of composition. The data are presented in Tables 5 to 8, and Figures 3 to 8, inclusive.

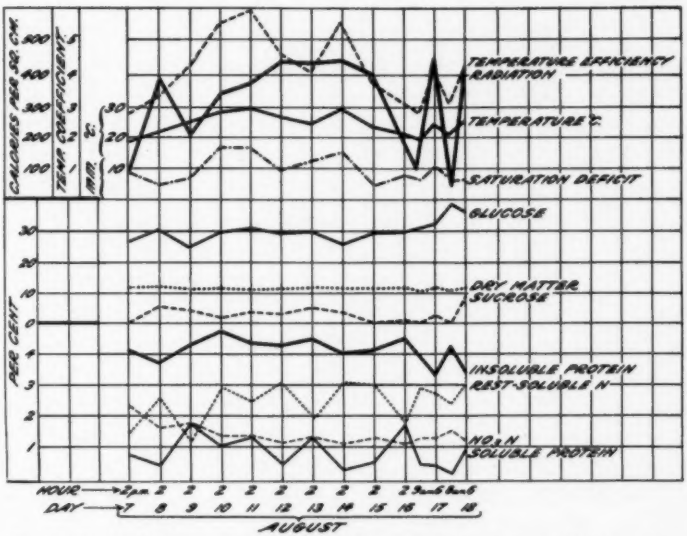

FIG. 4.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

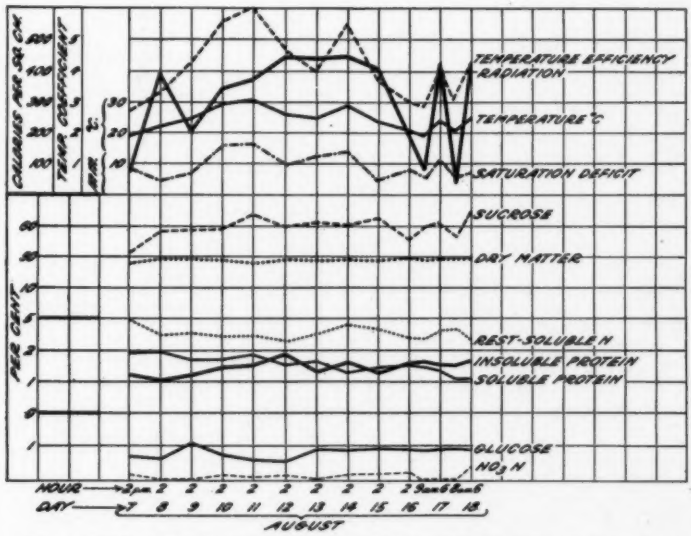

FIG. 5.—Graphs of climatic and analytical data of 1923 investigations of the root of sugar beet

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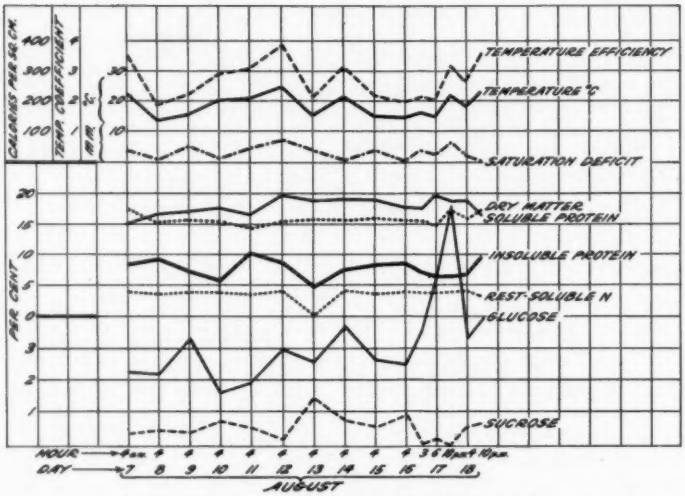

FIG. 6.—Graphs of climatic and analytical data of 1923 investigations of the leaf of sugar beet

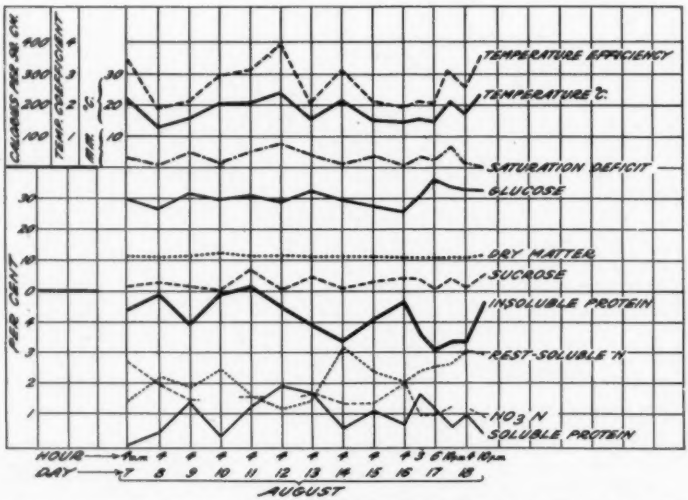

FIG. 7.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

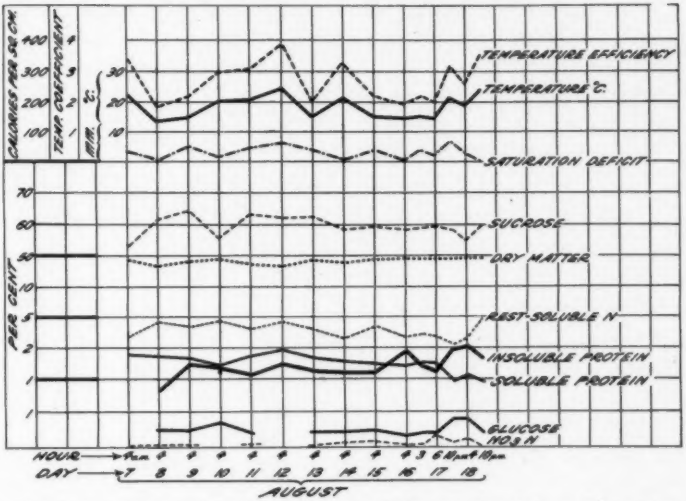

FIG. 8.—Graphs of climatic and analytical data of 1923 investigations of the roots of sugar beet

TABLE 5.—*Climatic data of sugar-beet sampling, 1923 (figs. 3 to 8)*

Date	Temperature	Temperature efficiency index	Vapor pressure deficit	Radiation, calories per square centimeter [2]	Date	Temperature	Temperature efficiency index	Vapor pressure deficit	Radiation, calories per square centimeter
	°C.		Mm.			°C.		Mm.	
Aug. 7, 4 a. m.	22.8	3.6	2.9	-------	Aug. 15, 4 a. m.	15.6	2.2	3.6	-------
2 p. m.	18.9	2.7	8.6	73	2 p. m.	23.3	3.7	4.5	403
Aug. 8, 4 a. m.	13.3	1.9	.9	-------	Aug. 16, 4 a. m.	13.9	1.9	.5	-------
2 p. m.	22.2	3.4	4.4	397	2 p. m.	20.6	3.1	8.5	207
Aug. 9, 4 a. m.	15.6	2.2	5.3	-------	Aug. 17, 3 a. m.	15.6	2.2	3.2	-------
2 p. m.	25.6	4.3	7.4	209	6 a. m.	14.4	2.0	2.3	-------
Aug. 10, 4 a. m.	20.0	2.9	1.7	-------	9 a. m.	19.4	2.8	5.7	90
2 p. m.	29.4	5.7	16.8	339	6 p. m.	25.0	4.2	10.3	435
Aug. 11, 4 a. m.	20.6	3.1	5.2	-------	10 p. m.	21.1	3.2	6.9	-------
2 p. m.	30.6	6.1	17.3	373	Aug. 18, 4 a. m.	18.3	2.6	2.0	-------
Aug. 12, 4 a. m.	23.9	3.9	7.1	-------	8 a. m.	20.6	3.1	5.4	40
2 p. m.	26.7	4.7	9.4	450	6 p. m.	25.6	4.3	6.8	411
Aug. 13, 4 a. m.	15.0	2.1	3.8	-------	10 p. m.	22.8	3.6	.0	-------
2 p. m.	24.4	4.0	12.3	434					
Aug. 14, 4 a. m.	21.1	3.2	.9	-------					
2 p. m.	29.4	5.7	14.3	447					

TABLE 6.—*Analytical data of sugar-beet leaf, 1923 (figs. 3 and 6)*

Date	Dry matter	Composition of dry matter				
		Insoluble protein	Soluble protein	Rest-soluble N×5	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m.	17.5	8.1	15.0	3.3	2.3	0.3
2 p. m.	15.7	4.9	20.0	3.0	2.9	.2
Aug. 8, 4 a. m.	15.4	9.0	16.8	2.8	2.1	.4
2 p. m.	19.0	6.2	18.1	3.1	4.8	1.2
Aug. 9, 4 a. m.	16.0	7.5	17.3	2.9	3.3	.3
2 p. m.	17.9	8.6	17.4	2.6	3.8	1.0
Aug. 10, 4 a. m.	15.5	5.6	17.8	3.4	1.6	.7
2 p. m.	18.0	7.8	16.5	3.3	1.4	1.9
Aug. 11, 4 a. m.	14.0	10.0	16.1	3.0	1.9	.5
2 p. m.	17.8	9.7	15.4	3.0	2.8	1.4
Aug. 12, 4 a. m.	15.4	7.9	19.2	3.7	3.0	.2
2 p. m.	18.4	4.8	19.1	3.6	4.3	.5
Aug. 13, 4 a. m.	15.9	4.3	18.3	.2	2.6	1.4
2 p. m.	19.2	6.9	15.3	4.5	4.7	1.3
Aug. 14, 4 a. m.	15.6	7.1	18.9	3.6	3.7	.7
2 p. m.	19.4	7.4	17.0	3.2	3.8	.0
Aug. 15, 4 a. m.	16.1	8.0	18.3	3.4	2.6	.5
2 p. m.	18.5	7.5	17.5	3.1	4.6	1.1
Aug 16, 4 a. m.	15.5	8.2	17.6	3.5	2.4	.5
2 p. m.	18.4	9.9	15.9	2.8	4.4	1.1
Aug. 17, 3 a. m.	15.5	7.0	17.6	3.5	3.6	.0
6 a. m.	14.6	6.2	19.7	3.4	5.1	.2
9 a. m.	17.1	11.8	14.4	2.8	4.0	.9
6 p. m.	18.2	9.8	14.1	3.0	4.3	.5
10 p. m.	17.1	6.5	18.3	3.7	7.6	.0
Aug. 18, 4 a. m.	16.3	6.8	18.8	3.8	3.2	.5
8 a. m.	17.5	7.6	18.4	3.5	5.0	.0
6 p. m.	17.8	8.6	16.6	3.5	4.2	1.1
10 p. m.	17.3	9.9	16.6	3.1	4.0	.6

TABLE 7.—*Analytical data of sugar-beet petiole, 1923 (figs. 4 and 7)*

Date	Dry matter	Composition of dry matter					
		Insoluble protein	Soluble protein	Rest-soluble N×5	NO_3	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m.	11.7	4.4	0.1	1.5	2.6	30.0	1.7
2 p. m.	12.2	4.1	.7	1.5	2.4	27.0	1.0
Aug. 8, 4 a. m.	11.5	4.9	.5	2.2	2.0	26.3	2.9
2 p. m.	12.7	3.8	.4	2.6	1.6	30.4	6.4
Aug. 9, 4 a. m.	11.9	3.9	1.5	1.8	1.6	31.3	1.7
2 p. m.	12.1	4.3	1.8	1.2	1.8	25.8	5.4
Aug. 10, 4 a. m.	12.9	4.9	.3	2.5	----------	29.2	.0
2 p. m.	12.2	4.8	1.0	2.9	1.3	29.6	2.4
Aug. 11- 4 a. m.	11.2	5.2	1.3	1.6	1.6	30.8	7.2
2 p. m.	11.9	4.4	1.3	2.5	1.4	31.1	4.4
Aug. 12, 4 a. m.	12.0	4.4	1.9	1.2	----------	28.5	.6
2 p. m.	12.2	4.3	.4	3.1	1.2	29.8	3.2
Aug. 13, 4 a. m.	11.4	3.9	1.4	1.6	1.7	32.8	5.1
2 p. m.	12.5	4.6	1.3	1.8	1.4	30.0	6.0
Aug. 14, 4 a. m.	11.2	3.4	.5	3.2	1.3	29.8	1.4
2 p. m.	12.4	4.1	.3	3.2	1.2	26.3	3.7
Aug. 15, 4 a. m.	11.4	4.1	1.1	2.3	1.4	28.5	3.0
2 p. m.	11.8	4.1	.6	3.0	1.3	29.6	.4
Aug. 16, 4 a. m.	11.2	4.7	.6	1.9	2.0	26.8	4.7
2 p. m.	12.0	4.6	1.8	1.8	1.1	29.8	1.6
Aug. 17, 3 a. m.	11.2	3.5	1.6	2.4	1.0	31.3	4.3
6 a. m.	10.9	3.0	1.1	2.6	1.0	36.6	.1
9 a. m.	11.1	3.9	.5	2.8	1.3	31.1	.0
6 p. m.	12.2	3.3	.4	2.7	1.3	32.8	2.7
10 p. m.	11.2	3.3	.7	2.7	1.3	34.1	3.7
Aug. 18, 4 a. m.	11.0	3.3	.9	3.1	----------	32.3	1.5
8 a. m.	11.3	4.3	.2	2.4	1.6	38.7	.0
6 p. m.	11.9	3.4	.9	3.0	1.2	26.1	9.6
10 p. m.	12.0	4.6	.3	2.9	0.9	32.2	5.3

TABLE 8.—*Analytical data of sugar-beet root, 1923 (fig. 5 and 8)*

Date	Dry matter	Composition of dry matter					
		Insoluble protein	Soluble protein	Rest-soluble N×5	NO_3	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m.	18.4		1.8	2.4	0.0		53.3
2 p. m.	18.0	1.1	1.9	2.9	.2	0.7	51.9
Aug. 8, 4 a. m.	17.4	0.6	1.8	2.9	.0	.5	63.0
2 p. m.	18.6	1.0	1.9	2.5	.0	.6	58.1
Aug. 9, 4 a. m.	17.9	1.6	1.7	2.7	.1	.5	64.7
2 p. m.	18.5	1.3	1.7	2.6	.0	1.1	59.2
Aug. 10, 4 a. m.	18.6	1.3	1.3	2.9		.6	56.9
2 p. m.	18.2	1.5	1.7	2.4	.2	.7	59.0
Aug. 11, 4 a. m.	17.3	1.1	1.8	2.6	.1	.4	63.0
2 p. m.	17.6	1.6	1.8	2.4	.1	.6	63.3
Aug. 12, 4 a. m.	16.9	1.5	1.9	2.9			61.5
2 p. m.	18.3	1.9	1.6	2.3	.2	.5	60.0
Aug. 13, 4 a. m.	17.8	1.2	1.7	2.6	.0	.4	61.8
2 p. m.	18.3	1.3	1.7	2.5	.04	.9	61.4
Aug. 14, 4 a. m.	17.6	1.2	1.6	2.3	.2	.4	58.5
2 p. m.	18.7	1.6	1.3	2.8	.2	.9	60.0
Aug. 15, 4 a. m.	18.2	1.2	1.4	2.8	.2	.4	59.3
2 p. m.	18.7	1.3	1.4	2.7	.2	.9	62.5
Aug. 16, 4 a. m.	18.8	1.8	1.4	2.3	.04	.3	58.0
2 p. m.	19.5	1.6	1.6	2.4	.2	.9	55.1
Aug. 17, 3 a. m.	17.9	1.4	1.6	2.5	.2	.4	58.6
6 a. m.	18.1	1.2	1.5	2.4	.4	.4	59.6
9 a. m.	18.9	1.7	1.4	2.4	.0	.8	59.6
6 p. m.	19.1	1.5	1.3	2.6	.0	.9	60.9
9 p. m.	18.4	2.0	0.9	2.1	.2	.8	58.1
Aug. 18, 4 a. m.	18.6	2.1	1.1	2.3	.3	.8	55.0
8 a. m.	19.2	1.5	1.1	2.7	.04	.9	56.0
6 p. m.	19.2	1.6	1.1	2.2	.4	.8	64.0
10 p. m.	19.3	1.7	0.9	3.1	.0	.4	60.0

Computations were made of the coefficients of correlation between individual climatic factors and various plant constituents. In only one case, however, was a significant relation found—namely, soluble protein of the leaf showed a negative correlation with temperature of $r = -0.390 \pm 0.106$. It is not surprising that in the case of the sugars at least, the averaging of values for periods of light with those for darkness failed to show correlations. One might well anticipate that synthetic effects of the former period would be offset by assimilative processes of the latter. In view of these conditions the graphs have been divided, as for the data of preceding seasons, into periods corresponding to light and darkness.

EFFECTS IN DAYLIGHT

EFFECTS IN THE LEAF

It seems that one of the most important features of the present investigation of the leaf concerns reducing sugars. The values for afternoon samplings show (fig. 3) that the percentage of reducing sugars expressed as glucose follow, in general, the variations of solar radiation. The depressions of reducing sugars on the 10th and 14th days, when radiation was at a high level, are associated with marked increased of the temperature efficiency. These relations seem to indicate that temperatures approaching 30° C. serve to reduce the apparent synthetic effects of a high plane of illumination. Comparison with the data for 1919 (fig. 1) indicates that radiation is here producing its maximum effect, and that somewhat under 5 per cent of reducing sugars may represent the maximum accumulation by this species of plant under these particular climatic conditions. This agrees with Campbell's values from the

sugar mangold (*6*), and those of Davis, Daish, and Sawyer obtained in the warm weather of August (*9*). The latter, however, found up to 12 per cent of hexoses in the cool weather of October.

Sucrose varied irregularly. Over a considerable portion of the period investigated it followed the course of reducing sugars, but at other times, especially from August 9 to 12, it varied inversely as the latter sugar. In view of these results it appears that glucose, as the preponderant form of reducing sugars, fluctuates more like a primary product of carbon assimilation than does sucrose.

The soluble protein in the leaf blades varied distinctly in a direction inverse to temperature. This is in agreement with the results of the previous seasons. Insoluble protein varied irregularly, but, with the exception of the period August 14 to 17, it fluctuated parallel to temperature, and hence inversely to soluble protein. This suggests a relation logically to be expected—namely, interconversion between soluble and insoluble protein. It appears significant, also, that insoluble protein varies inversely as reducing sugars, suggesting the possibility of the sequence: Hexose—soluble protein—insoluble protein. The relatively slight variations of rest-soluble nitrogen are also in the direction of those for reducing sugars, suggesting a direct relation of such carbohydrates to synthesis of these forms of nitrogenous compounds.

As the dry matter is relatively constant and shows no pronounced effects of saturation deficit of the air, it may be concluded that the latter factor had little influence in producing the effects analyzed here.

EFFECTS IN THE PETIOLE

In the petiole the variations of dry matter are insignificant, the effects of the saturation deficit of the air appearing to be negligible. The percentage of reducing sugars shows relatively little variation, but there is a distant correspondence to variations of reducing sugars in the leaf. It is partially related in the inverse direction to temperature changes. The small amounts of sucrose vary roughly in the same direction as reducing sugars. They therefore correlate with the monosaccharides of the leaf and not with its sucrose content. It appears from the data that invert sugar is the migratory form of carbohydrates, as generally observed by others.

The soluble protein in the petiole varies irregularly, but seems to be inversely related to radiation. Insoluble protein is generally variable with the soluble form. The rest-soluble nitrogen is distinctly related inversely to soluble protein. It appears to be positively related to temperature, hence varying inversely to the fluctuations of reducing sugars. Nitrate shows an inverse relation to reducing sugars and to rest-soluble nitrogen, while largely parallel to soluble protein. It thus appears that NO_3 is transformed to rest-soluble forms of nitrogen as hexoses accumulate, but that the soluble and insoluble proteins diminish simultaneously.

EFFECTS IN THE ROOT

Variations of the dry matter in the root are negligible and incapable of modifying metabolism. The percentage of reducing sugars varies appreciably in the forepart of the period of observation, as if depressed by rising temperature, but it is almost constant later, despite sharp variations of both temperature and illumination. Sucrose shows a distinct correlation with radiation. As

might be expected in a storage organ highly charged with this reserve, the variations were proportionately much less than those of the leaf. This constituent seems to follow primarily the variations of reducing sugars of both leaf and petiole, rather than those of sucrose therein.

Soluble protein shows here a response generally inverse to temperature changes, while insoluble protein varied relatively directly with this factor. Where there are disagreements, as on August 11 to 13, the response seems to be delayed. The rest-soluble nitrogen varies irregularly. Aside from August 14 to 17, it appears to be inversely related to temperature, and hence correlated with the formation of soluble protein. The variations of nitrate are suggestive of an inverse relation to reducing sugars in the fore period and to rest-soluble nitrogen later. However, the amount is too small to be interpreted as being of significance, especially in view of the possible complication from equilibrium in the root between nitrate diffusing backward from the petiole and that undergoing translocation from the soil.

EFFECTS IN DARKNESS

EFFECTS IN THE LEAF

With the exception of August 9 and the afternoon hours of August 17, the percentage of reducing sugars in the leaf varies with temperature. The marked aberrations of August 9 and excessive effect of the evening of August 17 may well be due to the high illumination of the preceding daylight period. Sucrose varies consistently in a manner inverse to temperature, and hence inversely to reducing sugars. Variations of soluble protein show a tendency to follow temperature at the fore period of growth, but are not distinctly correlated therewith. With the exception of August 13 and 14, insoluble protein varies in a manner inverse to temperature changes. Thus, the proteins of the leaf tend to show by night a temperature response inverse to that of the daytime. The rest-soluble nitrogen shows only one distinct variation (on August 13), and that is parallel to the changes of temperature.

EFFECTS IN THE PETIOLE

The reducing sugars in the petiole vary irregularly with reference to temperature. Sucrose behaves similarly. Excepting the dates from August 11 to 13, soluble protein varied in an inverse relation to temperature. Insoluble protein also varied chiefly in the above manner. Thus these compounds show the same trend in the petiole as by day. The rest-soluble nitrogen varies quite irregularly. Nitrate trends toward an inverse relation to temperature. It therefore shows a rather distinct inverse relation to rest-soluble nitrogen and to reducing sugars.

EFFECTS IN THE ROOT

The percentage of reducing sugars in the root shows no correlation with climatic factors. Sucrose varies in a manner inverse to temperature changes. The latter sugar, therefore, follows its own variations in the leaf. Soluble protein shows a somewhat inverse relation to temperature, and the same is true of insoluble protein. Rest-soluble nitrogen is also irregular, but with a tendency to follow the course of temperature. The slight amounts and variations of nitrate can not be considered significant.

Of the preceding particulars, the following relations appear to be most definite: During exposure to daylight the reducing sugars increase in the leaf with illumination, when the temperature remains well below 30° C. Rest-soluble nitrogen varies parallel to reducing sugars. The relative amounts of these various compounds suggest the sequence: Reducing sugars—amino acids—soluble proteins—insoluble proteins. Simultaneously, the sugars of the petiole are determined somewhat by the variations of reducing sugar in the leaf, but the proteins show little correlation with climatic factors. The variations of nitrates and rest-soluble nitrogen are such as to indicate the sequence: Reducing sugars—rest-soluble nitrogen. Sucrose in the root responds to the variations of reducing sugars in leaf and petiole. Protein is stored in insoluble form at higher temperatures. In general, the results show the delayed response to be expected between leaf and root.

In darkness, the reducing sugars of the leaf increase at the expense of sucrose as the temperature rises. Simultaneously, the proteins also undergo hydrolysis and migrate to the petiole. In the latter organ the same status of proteins is maintained, indicating further migration of these compounds to the root at higher temperatures. The variations of nitrate in the petiole suggest that these, in conjunction with reducing sugars, are converted to rest-soluble forms, although this synthetic process may occur in the leaf blade. Sucrose in the root shows an inverse response to temperature during darkness, but proteins are less definitely influenced.

DISCUSSION

Since the early investigations of Brown and Morris, the nature of the first sugar produced in carbon assimilation of chlorophyllous plants has been a mooted question. These workers worked with leaves of nasturtium dried in a steam-heated oven. Fortunately, this tissue dries rapidly, so that the chance of caramelizing is relatively low. The analytical methods employed were the most reliable then extant. As a result of marked accumulation of sucrose during illumination by sunlight, while the reducing sugars varied irregularly, Brown and Morris (*2*) inclined to the belief that the former was the first sugar of photosynthesis. Parkin (*15*) followed the distribution of sugars in the leaf of the snowdrop (*Galanthus nivalis*). On exposure of etiolated plants and of detached leaves to daylight, sucrose increased very decidedly while the percentage of hexoses remained rather constant. The ratio of sucrose to hexoses decreased from the tip of the leaves toward the base, and increased as the season advanced. Parkin suggests it is quite possible that sucrose is the first tangible product of carbon assimilation.

Strakosch (*21*) observed, by means of the osazone reaction, that glucose seemed to be the first sugar formed in the mesophyll of the sugar-beet leaf. The reduction test with Fehling's solution upon tissue extracts showed a great preponderance of hexoses over sucrose in the leaf. Upon exposure to sunlight after prolonged etiolation the hexoses of the leaf were rapidly converted to sucrose. Similar observations led Dixon and Mason (*10*) to suggest that hexoses are synthesized in the protoplasm, sucrose being condensed therefrom and excreted into the vacuoles.

Campbell (*6*), on the basis of reliable methods of extraction and analysis, but with admittedly too few data to be conclusive, obtained

evidence that an increase of hexoses precedes that of sucrose in leaves of the sugar mangold with the advent of daylight periods.

Davis, Daish, and Sawyer (*9*) present data for the distribution of carbohydrates in leaves of the mangold at intervals of two hours. These cover three days from August 26 to October 11. The variations of sucrose exceed those of the hexoses. On account of the low temperatures prevailing on their later dates of examination, their data (Table 9) are not comparable with those of the present writers. The results of August 26–27 are shown graphically in

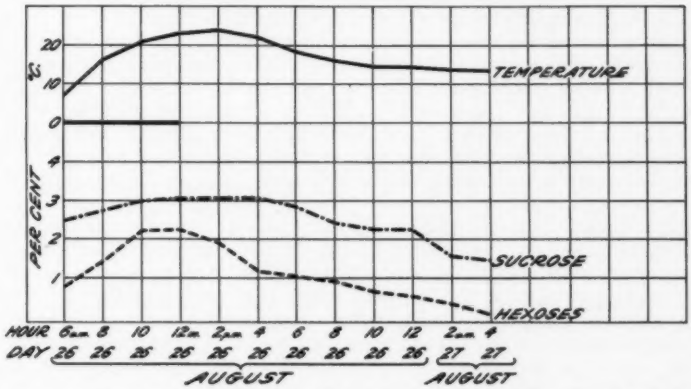

FIG. 9.—Graphs illustrating data of Davis, Daish, and Sawyer on distribution of carbohydrates in leaf of the mangold at intervals of two hours, Aug. 26–27

Figure 9. The percentage of hexoses declined after 10 a. m., when the temperature had reached 21°C. The total sugars also attained a maximum value by 10 a. m., and this plane was maintained until toward 2 p. m. Because of the generally greater variation of hexoses than of sucrose, these investigators consider that their results support the conception of the latter sugar as the primary one of carbon assimilation. As to the relative response of the sugars to the change from darkness to daylight, their results are inconclusive.

TABLE 9.—*Data from Davis, Daish, and Sawyer for Figure 9*

Date	Temperature	Hexose	Sucrose	Date	Temperature	Hexose	Sucrose
	°C.	*Per cent*	*Per cent*		°C.	*Per cent*	*Per cent*
Aug. 26, 6 a. m.	7.2	0.8	2.5	Aug. 26, 6 p. m.	18.9	1.0	2.8
8 a. m.	16.8	1.4	2.8	8 p. m.	16.1	.9	2.4
10 a. m.	21.1	2.2	3.0	10 p. m.	14.7	.9	2.2
12 a. m.	22.9	2.2	3.1	12 p. m.	14.4	.6	2.2
2 p. m.	23.9	1.9	3.1	Aug. 27, 2 a. m.	13.9	.4	1.6
4 p. m.	21.7	1.2	3.1	4 a. m.	13.3	.2	1.5

The accumulation of sucrose while hexoses are decreasing possibly may be explained by the respiratory effect of relatively high temperatures. Brown and Morris (*2*) estimated that glucose was utilized to a greater extent than fructose in respiration. Lindet (*12*) grew the embryo of barley on invert sugar, and found a utilization of 17 to 70 per cent more glucose than fructose. If this is true generally,

one can understand what prompted Boysen-Jensen (*1*) to make his observation on the accumulation of sucrose in germinating peas. If fructose accumulates, even though the plane of glucose is reduced, it would seem quite possible for the condition of equilibrium between these sugars to induce the formation of sucrose.

Thoday (*23*) quotes Broocks' results from weighings of leaf sections which indicate the cessation of photosynthesis at midday. Thoday ascribed this to engorgement of the leaf tissue with photosynthetic products. It would seem, recognizing limitations of Broocks' method of measurement and his unknown temperature relations, that the effect here discussed might have been due to an excess effect of respiration over synthesis. Spoehr (*20, pt. 2*) has shown the interrelation between respiration and photosynthesis of carbohydrates in leaves of the bean and sunflower. He found that an increase of photosynthesis definitely stimulated respiration. It appears that this effect must be taken into account in interpreting the results of others cited here and those of the present writers which indicate that high temperatures cause a depression in the percentage of reducing sugars in leaf-blade tissue. Moreover, if one considers the criticism of Brown and Heise (*3, 4*), the rate of carbon assimilation is not augmented much by an increase of temperature and it decreases with increasing illumination. Thus, in the writers' results for 1923 the overstepping of the most efficient light intensity may have operated in conjunction with higher temperatures to produce the limited content of reducing sugar attained. The writers interpret their results as indicating that these sugars are among the primary products appearing in carbon assimilation.

The writers recognize the claim of Chibnall (*7*) that computations based upon fresh weight of tissue overcome errors incident to determinations based upon dry weight. Nevertheless, they are unable to comprehend how the former method of calculation could alter the proportions of constituents when the moisture content fluctuates little. It is likewise difficult to conceive how allowance for large variations in moisture content could compensate differences in the rate of either synthesis or translocation of constituents in the leaf dry matter. Even the method of determining diurnal changes in tissue components by computation of absolute amounts per individual leaf can not be considered reliable, because of the possible removal or alteration of compounds contemporaneously with synthesis of the same. The latter factors might be compensated in part by includiug analyses of the other organs of the plant with those of the leaf. When computed to the basis of fresh weight of tissue, the writers' data of Table 6 show a corresponding increase of sugars in the afternoon. None of the relative diurnal values for other constituents are altered, except in the case of soluble protein, in which case the percentage is higher in the afternoon than in the morning on 9 of the 12 dates. As shown by their preceding statements, however, the writers do not consider this relation final proof of maximum synthesis of protein in daylight. In view of the limitations imposed upon separation of products of catabolism from those of anabolism, it seems necessary to consider simultaneous changes of composition in different organs of the plant. This situation greatly complicates the problem considered here. The writers have attempted to deal with it in the preceding survey of their results.

The results reported here give promise that it will be possible to determine the effects of specific climatic factors upon metabolism by means of chemical analysis of the plant tissue at frequent intervals, accumulating sufficient data to justify the computation of correlation coefficients. Perhaps conclusive results can be obtained in this way as surely as by the use of special equipment for the control of environmental factors, more particularly of temperature, atmospheric humidity, and illumination. The capacity of this type of equipment for plant yields must remain quite limited, except at exorbitant cost. Even when results have been tested under such artificial conditions, there remains the problem of determining to what extent the effects discovered obtain under the climatic complex to which field practice is subject.

SUMMARY

Results are presented for diurnal changes of chemical composition in leaf blades of the sugar mangold and sugar beet, and in the petiole and root of the latter plant.

The percentage of reducing sugars in the leaf blade increases with solar radiation, within limits. Temperature seems to be a limiting factor in the increase of these sugars when its value approaches 30° C.

The percentage of soluble protein in the leaf varies in a manner inversely related to temperature and therefore correlated with the fluctuation of reducing sugars. This constituent gave a correlation with temperature of -0.390 ± 0.106 for periods of light and darkness combined.

The foliar fluctuations of reducing sugars are distantly paralleled by the deposition of sucrose in the root, while relatively high temperatures increase the percentage of protein stored in this organ.

The relations in the petiole are such as to suggest that a high plane of reducing sugars in the plant, together with the presence of nitrates, leads to the formation of amino acids and rest-soluble nitrogen.

These results explain practical observations that cool, fair weather, such as that common to the autumn season, is favorable to the storing of high percentages of sugar in the root of the sugar beet.

LITERATURE CITED

(1) Boysen-Jensen, P.
1912. Über synthetische vorgänge im pflanzlichen organismus. I. die rohrzuckersynthese. Biochem. Ztschr. 40: 420–440, illus.

(2) Brown, H. T., and Morris, G. H.
1893. A contribution to the chemistry and physiology of foliage leaves. Jour. Chem. Soc. [London] 63: 604–677.

(3) Brown, W. H., and Heise, G. W.
1917. The application of photochemical temperature coefficients to the velocity of carbon dioxide assimilation. Philippine Jour. Sci. (C) 12: 1–25, illus.

(4) ——— and Heise, G. W.
1917. The relation between light intensity and carbon dioxide assimilation. Philippine Jour. Sci. (C) 12: 85–97, illus.

(5) Browne, C. A.
1912. A handbook of sugar analysis. 787 p., illus. New York and London.

(6) Campbell, A. V.
1912. Carbohydrates of the mangold leaf. Jour. Agr. Sci. 4: 248–259, illus.

(7) CHIBNALL, A. C.
1923. DIURNAL VARIATIONS IN THE TOTAL NITROGEN CONTENT OF FOLIAGE LEAVES. Ann. Bot. [London] 37: 511–518.

(8) ———
1924. INVESTIGATIONS ON THE NITROGENOUS METABOLISM OF THE HIGHER PLANTS. PART V. DIURNAL VARIATIONS IN THE PROTEIN NITROGEN OF RUNNER BEAN LEAVES. Biochem. Jour. 18: 387–394.

(9) DAVIS, W. A., DAISH, A. J., and SAWYER, G. C.
1916. STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS. I. THE CARBOHYDRATES OF THE MANGOLD LEAF. Jour. Agr. Sci. 7: 255–326, illus.

(10) DIXON, H. H., and MASON, T. G.
1916. THE PRIMARY SUGAR OF PHOTOSYNTHESIS. Nature [London] 97: 160.

(11) KOSUTANY, T.
1897. UNTERSUCHUNGEN ÜBER DIE ENTSTEHUNG DES PFLANZENEIWEISSES. Landw. Vers. Sta. 48: 13–32.

(12) LINDET, L.
1911. SUR LE POUVOIR ÉLECTIF DES CELLULES VÉGÉTALES VIS-À-VIS DU DEXTROSE ET DU LÉVULOSE. Bul. Soc. Chim. France (4) 9: 425–429.

(13) LINK, K. P., and TOTTINGHAM, W. E.
1923. EFFECTS OF THE METHOD OF DESICCATION ON THE CARBOHYDRATES OF PLANT TISSUE. Jour. Amer. Chem. Soc. 45: 439–447.

(14) OSBORNE, T. B., WAKEMAN, A. J., and LEAVENWORTH, C. S.
1921. THE PROTEINS OF THE ALFALFA PLANT. Jour. Biol. Chem. 49: 63–91.

(15) PARKIN, J.
1911. THE CARBOHYDRATES OF THE FOLIAGE LEAF OF THE SNOWDROP (GALANTHUS NIVALIS, L.), AND THEIR BEARING ON THE FIRST SUGAR OF PHOTOSYNTHESIS. Biochem. Jour. 6: 1–47, illus.

(16) PIGORINI, L.
1914. STUDÎ SULLA FOGLIA DI GELSO: SULLA COMPOSIZIONE CHIMICA DELLA FOGLIA AL MATTINO E ALLA SERA. Atti. R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat. (5) 23 (2): 433–437.

(17) RUSSELL, H. L., and MORRISON, F. B.
1920. NEW FARM FACTS. Wis. Agr. Exp. Sta. Bul. 323, 98 p., illus.

(18) SCHULZE, B., and SCHÜTZ, J.
1909. DIE STOFFWANDLUNGEN IN DEN LAUBBLÄTTERN DES BAUMES, INSBESONDERE IN IHREN BEZIEHUNGEN ZUM HERBSTLICHEN BLATTFALL. Landw. Vers. Sta. 71: 299–352.

(19) SHAFFER, P. A., and HARTMANN, A. F.
1921. THE IODOMETRIC DETERMINATION OF COPPER AND ITS USE IN SUGAR ANALYSIS. I. EQUILIBRIA IN THE REACTION BETWEEN COPPER SULFATE AND POTASSIUM IODIDE. II. METHODS FOR THE DETERMINATION OF REDUCING SUGARS IN BLOOD, URINE, MILK, AND OTHER SOLUTIONS. Jour. Biol. Chem. 45: 349–390, illus.

(20) SPOEHR, H. A., and MCGEE, J. M.
1923. STUDIES IN PLANT RESPIRATION AND PHOTOSYNTHESIS. 98 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 325.)

(21) STRAKOSCH, S,
1907. EIN BEITRAG ZUR KENNTNIS DES KOHLENHYDRATSTOFFWECHSELS VON BETA VULGARIS (ZUCKERRÜBE). Sitzber. K. Akad. Wiss. [Wien], Math. Naturw. Kl. (I) 116: 855–869.

(22) SUZUKI, U.
1897–98. ON AN IMPORTANT FUNCTION OF LEAVES. Bul. Col. Agr., Tokyo Imp. Univ. 3: 241–252.

(23) THODAY, D.
1910. EXPERIMENTAL RESEARCHES ON VEGETABLE ASSIMILATION AND RESPIRATION. VI. SOME EXPERIMENTS ON ASSIMILATION IN THE OPEN AIR. Proc. Roy. Soc. [London] (B) 82: 421–450, illus.

(24) TOTTINGHAM, W. E., SCHULZ, E. R., and LEPKOVSKY, S.
1924. THE EXTRACTION OF NITROGENOUS CONSTITUENTS FROM PLANT CELLS. Jour. Amer. Chem. Soc. 46: 203–208.

DECOMPOSITION OF ORGANIC MATTER IN SOIL[1]

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INTRODUCTION

The chemical and biological decomposition of organic matter in the form of green manure when incorporated with the soil is of fundamental importance in improving soil conditions and plant growth. Green manuring is an important means of restoring soil fertility, and is especially applicable to Virginia agriculture, as most of the soil types in Virginia are very deficient in organic matter. This subject is of great economic importance, and the writer believes that further investigation of the effects of green manures on soils is needed.

Former work (*3*)[2] on this subject at the Virginia Agricultural Experiment Station has shown that when such crops as blue grass, soy beans, rye straw, and clover are incorporated with the soil their organic nitrogen rapidly passes over into nitrates, and that the bacterial flora of the soil is improved by such treatment. In addition to these facts it has been shown by experiment that plant growth has been materially stimulated by the addition of certain green materials to the soil.

Another phase of green manuring that has received attention at this station is the so-called acidity resulting from the turning under of green crops. Results showed that the general belief among farmers that decomposing organic matter in the soil produced acid conditions which are injurious to the growth of crops was not warranted. There was a slight acidity in the early stages of growth, but the acidity resulting from the turning under of the very tender grasses was not permanent. The general tendency was toward the creation of an alkaline state rather than an acid one, and if proper consideration is given to fertilizer treatment which contains a place for lime it seems that no harm results from green manuring.

The phase of the subject with which the subsequent experiments are concerned takes into consideration the stage of growth of the green manure crop in relation to its effectiveness when incorporated with the soil.

Rye is a much used green-manure crop. This plant varies widely in chemical composition between April 15 and June 15. Not only does the composition vary, but succulency diminishes as growth advances, and one would expect the rate of decomposition in the soil to be slower the nearer the crop is to maturity when turned under.

In studying the chemical composition of green-manure crops through successive stages of their growth, it will be seen that as the

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[2] Reference is made by number (italic) to "Literature cited," p. 98.

nitrogen of the plant diminishes the crude fiber increases, therefore one would expect the younger cuttings to contain the relatively greater amount of nitrogen and the lesser amount of crude fiber. As this gap between the nitrogen and crude fiber widens, there is reason to believe that the change will have its effect on the rate of decomposition when the green manure is turned into the soil.

In former experiments (*3*) it has been proved that cellulose or woody fiber, in a pure state, decomposes very slowly in the soil. If green-manure crops may be likened to impure forms of cellulose, enriched by various substances, a study of the pure and impure forms should throw some light on the very important practice of green manuring.

It is hoped that the results presented here will give some idea as to the best time to turn under green crops so that they will have the maximum value in replenishing the soil's store of organic matter.

REVIEW OF PREVIOUS INVESTIGATIONS

It appears from the literature that scientific effort has been largely directed toward the chemical rather than the bacteriological side of the question. The bacterial life of the soil plays an important part in breaking up the complex organic compounds, in the formation of humus, and in the formation of nitrates so necessary for the plant's development.

The published work on green manuring has been of a very general nature, and very few investigators seem to have attempted to establish the exact time at which green organic matter should be turned into the soil to give the greatest aid to the many complex activities of the soil flora.

White (*12*), experimenting with clover as a green manure, found that the younger the crop when turned under the more rapidly it decomposed. He also found that there was a stimulating effect on a later tomato crop.

Hutchinson and Milligan (*4*) measured the decay of organic matter in the soil by the rate of nitrate accumulation. These investigators observed that nitrification diminished with the age of the green manures applied.

Baessler (*2*), investigating the value of paulines, serradella, crimson clover, and hairy vetch as green manure plants, in humus sandy soils, advised turning under as late as possible in the life of the plants, and not to turn under in the hot summer when the plants are green.

Pfeiffer (*8, p. 13*), in his studies on the nitrogen assimilating bacteria, found that the open sandy soils gave the best results when green manures were applied. The same investigator and three of his associates (*7, p. 733*) noted a harmful effect when straw was used as a green-manure crop.

Maynard (*5*), using the accumulation of nitrates as an indication of the rate of decomposition of sweet clover, found that the rate of decay of the sweet clover decreased as the plant became more mature.

Merkle (*6*), investigating the decomposition of organic matter in soils, used as a measure of decay the formation of humus and the evolution of carbon dioxide, and found that the more succulent the plant the more rapidly does it decay in the soil.

Starkey (*10*) found that the decomposition of organic matter in the soil was closely related to the evolved carbon dioxide, and that the evolution of this gas served as an index to the processes of decomposition. He experimented with cellulose, rye straw, alfalfa meal, fungous material, dried blood, and dextrose.

Waksman and Heukelekian (*11*) found that the determination of the power of a soil to decompose cellulose can be added to the group of methods used for carrying out a microbiological analysis of a soil. They suggested certain methods to measure the cellulose-decomposing power of the soil, each method yielding information viewed from a different angle.

Seelhorst (*9*) observed that when green manures are turned under in the fall more nitrogen is lost and less is found in the succeeding crop. This investigator found that the late turning under of green manure had many disadvantages, and in every case the nitrogen in the crop was greater with spring green manuring.

Whiting and Schoonover (*13*), in greenhouse and field experiments, compared the rate of decomposition of green and cured clover tops in soil, and found that the process of curing retarded the rate of decomposition, as measured by ammonification, nitrification, and loss of carbon. In commenting on the hypothetical reasons for the differences in rate of decomposition between green and cured clover, they say:

> In considering the greater initial rate of decomposition exhibited by the green clover as found by all methods used to study the decomposition, it did not appear that there were any indications of purely chemical changes having occurred in the clover during dehydration. Enzymic action as a factor in affecting the cell contents is not an impossibility, but the temperature at which the green clover was held during the dehydration of the lot with which it was compared, would exclude the possibility of much change in it, while the dehydration process with the other lot of clover would require the action of the synthetic enzymic processes to retard its rate of decomposition by stabilizing the nitrogen compounds.
>
> The explanation that seems to fit the results found is based on a physical hypothesis. The loss of water is of course accompanied with an increase in the concentration of salts in the cells. This brings about a hardening of the cell contents, and the whole material becomes more horny and shriveled. The diffusion of nitrogenous substances is stopped until hydration has been reestablished. The colloidal nature of the cell contents causes them to become hydrated slowly, after a previous dehydration. Apparently the time required to bring about the hydration in a normal soil is sufficiently long for the ammonifying bacteria to gain headway on the green material, and consequently to effect a much greater rate of decomposition than those acting on the dehydrated material.

In a previous work (*3*) the effect of cellulose was studied with respect to nitrification in several leading types of Virginia soil originating from distinct geological formations. The results showed a depression of nitrate in every case, and in many instances the nitrate already present in the soil was used up. There was a decided diminution in plant growth, with a yellow appearance in all the plants grown in a cellulose-containing medium. The following work was carried out with the object of determining the effect of cellulose on the plant when the nitrogen supply was in a very soluble form.

EFFECT OF CELLULOSE ON PLANT GROWTH IN THE PRESENCE OF NITROGEN IN A VERY SOLUBLE FORM

The soil selected for this work represented a typical Hagerstown silt loam, and came from an unfertilized plot that had been in corn for about eight years. The soil was practically exhausted as far

as nitrate nitrogen was concerned, for on analysis it showed only 0.6 milligram of nitrogen in each 100 grams of soil. A soil containing such a small amount of this substance is an ideal medium for study when enriched with organic matter. The pots used were of the Wagner pattern, with side tubes, and of 12-kilogram capacity. Corn was the crop used as the indicator.

In previous work on nitrate formation where green crops had been turned under, the calculations for the additions of cellulose were made on a 4-ton basis, or 35.5 grams per pot of 12-kilogram capacity. Taking these figures as a basis, the amount of cellulose was varied accordingly, beginning with an addition of cellulose representing 1 ton and increasing the amount to about 28 tons per acre.

In the first series of experiments the quantities of cellulose added to the soil varied, but no nitrate was added. The second series contained soils varying in cellulose, with the nitrate constant. A third series was run, varying both cellulose and nitrate. Where the nitrate was constant, one-hundredth of 1 per cent of potassium nitrate was added to the mixtures.

TABLE 1.—*Effect of cellulose on the growth of corn in pots, varying amounts of cellulose being added to Hagerstown silt loam soil but without the addition of any fertilizer materials*

Pot No.	Cellulose applied	Weight of plants produced		Pot No.	Cellulose applied	Weight of plants produced	
		Green	Dry			Green	Dry
	Gm.	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*
1	8.87	210.0	52.3	6	142.00	4.6	1.9
2	17.75	150.4	30.5	7	177.50	5.3	1.5
3	35.50	102.8	16.7	8	213.00	13.1	2.5
4	71.00	162.9	25.8	9	248.50	2.7	1.4
5	106.50	23.7	5.2				

It will be seen from Table 1 that apparently no marked injurious effect was shown by the addition of cellulose to the soil until quantities exceeding 71 grams (or the equivalent of 8 tons of organic matter per acre) had been reached. Above this amount the growth of the plants was restricted to a marked degree. In pot 9, receiving 248.50 grams of organic matter, plant growth almost stopped.

TABLE 2.—*Effect of cellulose and potassium nitrate on the growth of corn in pots, varying amounts of cellulose and a constant amount of potassium nitrate being added to Hagerstown silt loam*

Pot No.	Treatment		Weight of plants produced		Pot No.	Treatment		Weight of plants produced	
	Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry		Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry
	Gm.	*Per cent*	*Gm.*	*Gm.*		*Gm.*	*Per cent*	*Gm.*	*Gm.*
10	8.87	0.01	250.7	49.9	15	142.00	0.01	2.3	1.0
11	17.75	.01	142.4	21.2	16	177.50	.01	3.0	1.0
12	35.50	.01	86.4	15.0	17	213.00	.01	3.5	1.3
13	71.00	.01	27.3	3.4	18	248.50	.01	5.8	2.3
14	106.50	.01	7.7	1.4					

A constant quantity of nitrate failed to counteract the injurious effects of increasing quantities of cellulose added to the soil. The results in Table 2 duplicate those shown in Table 1 fairly closely. There is a gradual restriction of plant growth in all pots above the 8-ton-per-acre application, and the addition of 0.01 per cent potassium nitrate failed to counteract the depressing effect of the cellulose.

TABLE 3.—*Effect of cellulose and potassium nitrate on the growth of corn in pots, varying amounts of cellulose and potassium nitrate being added to Hagerstown silt loam*

Pot No.	Treatment		Weight of plants produced		Pot No.	Treatment		Weight of plants produced	
	Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry		Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry
	Gm.	*Per cent*	*Gm.*	*Gm.*		*Gm.*	*Per cent*	*Gm.*	*Gm.*
19	8.87	0.001	341.3	42.8	25	177.50	0.100	143.3	17.5
20	17.75	.003	366.1	38.5	26	213.00	.300	13.1	3.1
21	35.50	.008	256.2	37.7	27	248.50	.500	7.6	2.7
22	71.00	.009	330.0	33.5	28	Check.		283.2	68.6
23	106.50	.010	363.0	37.0	29	Check.		265.5	63.8
24	142.00	.050	295.3	33.5					

Varying the quantity of nitrate had very little effect on plant growth in the above series, except that the stimulating effect of the nitrate was carried a little further than in the former experiments. Plant growth in the former studies was diminished after 8 tons per acre of the organic matter had been added, but increasing the amount of nitrate tended to increase plant growth slightly beyond this stage. It is evident that keeping such apparently inert material as cellulose at about 1 ton per acre, and increasing the quantity of nitrate, will eliminate many of the ill effects of this substance. It is apparent in applying these results to substances containing a wide nitrogen-carbon ratio, such as mature rye and wheat straw, that additions of nitrate will hasten their decomposition and availability to the plant.

A striking fact is shown in Table 3 concerning the weight of the plants. Regardless of the quantities of potassium nitrate added (either the same or smaller or greater than in the preceding experiments) the weight of the plants was uniformly greater in this series than in the other series for corresponding quantities of cellulose applied, except for one green weight and one dry weight. All of the plants were cut at the same time and had reached the same stage of maturity, but the succulency of the crop grown in this experiment (even that part receiving very little nitrate) was greater than in the preceding experiments. This is a very desirable quality in certain crops, especially crops to be turned under as green manures, as subsequent experiments will tend to show that there is a definite stage of growth of green-manure crops which is associated with maximum decomposition, taking into account their chemical composition with reference to nitrogen.

In the above experiment the vigor and health of the plants were much improved over the series which had no nitrate and those receiving the nitrate in constant amount.

EFFECT OF CELLULOSE ON PLANTS GROWN IN A NUTRIENT SOLUTION

The foregoing experiments have shown that there is a diminution in growth and vigor of plants when the amount of the soil-incorporated cellulose is increased, and that the ill effect is proportional to the amount of cellulose added. The addition of potassium nitrate in varying quantities has had little effect in diminishing the effect of cellulose beyond a 4-ton application of the same.

A series of experiments was run to further study this question, cellulose being added to a nutrient solution. This medium was replaced by a fresh solution of the same composition from time to time in some cases, but in others the solution was not renewed and the effect on plant growth was noted.

The nutrient solution selected (Crone's) was of the following composition: Water 1,000 c. c.; potassium nitrate 1 gm.; iron phosphate (ferric) 0.5 gm.; calcium sulphate 0.25 gm.; magnesium sulphate 0.25 gm. It was prepared in 20-liter quantities.

Large stoneware vessels were used for growing the plants, and 35.5 gm. of cellulose was used to 3,000 c. c. of nutrient solution.

As a preliminary study four pots were used, two as checks, in which the nutrient solution was renewed three times each week, and two to which the equivalent of 4 tons of cellulose per acre had been applied and the nutrient solution renewed as in the case of the check pots. Figure 1 shows the effect of the treatments on the plants and their root systems.

Where the cellulose was eliminated the plants were healthy and vigorous and had a rich green color. With the cellulose-treated pots the plants were stunted in growth and had a very yellow, unhealthy appearance, even though the nutrient solution was replenished three times a week. The root systems of the plants receiving no cellulose were healthy and very white and clean, while those of the cellulose-treated pots were dark and gave off a slight odor of hydrogen sulphide. The dry weight of the plants from the check pots averaged 28.7 gm., while the average for the cellulose pots was 4.6 gm. The checks therefore contained more than six times the dry matter as was obtained from the cellulose-treated pots. Nitrogen determinations on these crops were not made, but former experiments (*3*) have shown a corresponding decrease in nitrogen for plants grown in soils that had been treated with cellulose. These experiments indicate that cellulose restricts plant growth, even though the medium in which the plant is grown is a well-balanced one in every particular.

As a continuation of this study the following series were run, the same nutrient solution being allowed to remain for the whole period in two instances, and it being changed at regular intervals in the others. The plan was as follows:

Pot No.	Nutrient solution	Treatment	Crop results, dry-weight basis
			Gm.
211	No cellulose	Renew nutrient solution once each week	43.6
210	do	No renewal of nutrient solution	18.4
8	Cellulose	Renew nutrient solution twice each week	10.7
15	do	Renew nutrient solution once each week	8.6
16	do	No renewal of nutrient solution	2.7

In the pots that did not receive the cellulose treatment the plants were vigorous and healthy (fig. 2, A). Changing the solution once each week gave an increase in plant growth amounting to 25.2 gm., dry weight.

When celluslose was added the plants were poor and the root system very much discolored (fig. 2, B). With pot 8, in which the

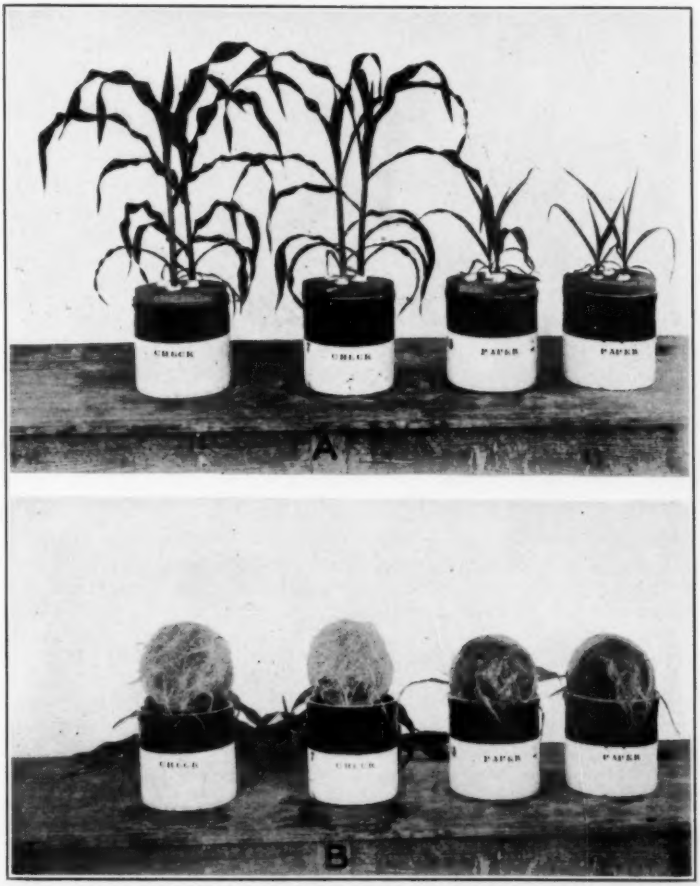

FIG. 1.—A. Showing the effect of cellulose on the growth of corn in pots, using Crone's nutrient solution. B. Root systems of the same plants

nutrient solution was renewed twice a week, there was a slight gain over pot 15, which had the nutrient solution renewed only once each week. Growth was very poor in pot 16, where there was no renewal of the nutrient medium.

There was a copious evolution of hydrogen sulphide in all of the cellulose pots after about four weeks. There was a sufficient quantity of this gas present to give a test with lead acetate. This evolu-

tion of hydrogen sulphide was probably caused by the action of the decomposing organic matter on the sulphates of calcium and magnesium present in the nutrient solution. Diminished growth results.

Apparently the ill effects produced by cellulose were as marked in the nutrient solution as when the plants were grown in soil with the same additions of cellulose.

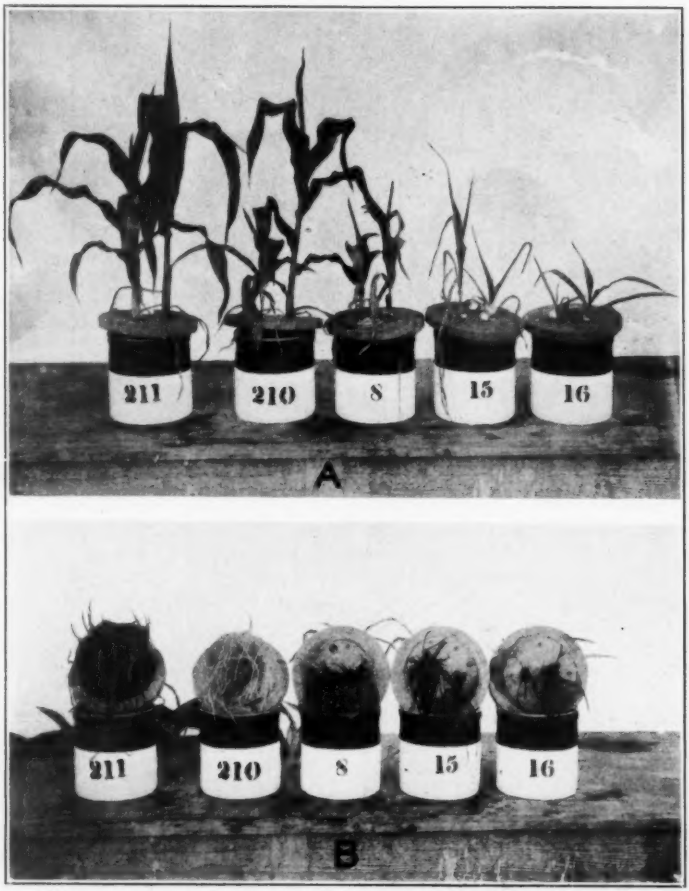

FIG. 2.—A, Showing the effect of cellulose on the growth of corn in pots, when the nutrient solution was renewed at different times. B Root systems of the same plants

Plants contain varying amounts of cellulose, depending more or less upon the other constituents present. In other words, the crude fiber contained in plants may be regarded as a more or less impure form of cellulose, and it is believed that the greater the amount of cellulose contained in the plant the slower will be its decomposition when incorporated with the soil. For instance, it is thought that

young rye cut about April 15 is more readily decomposed in the soil than rye cut on or about June 1, assuming that both were planted at the same time.

Former observations have shown that rye has a very peculiar effect on soil of certain types, there seeming to be a tendency toward restriction of plant growth; however, it is thought that if rye is cut at a certain stage of development some of the objectionable effects may be eliminated.

As a preliminary study of this question the following experiments were undertaken. Large wooden tubs, holding about 75 pounds of soil, were arranged for proper drainage and were kept out of doors. Crimson clover was added to soil and sand at the rate of 5 tons per acre. A typical Hagerstown silt loam from Blacksburg was used in the experiment. Corn was the indicator crop. The average results over a period of three years are given in Table 4.

TABLE 4.—*Effect of crimson clover on the growth of corn in tubs when added to Hagerstown silt loam*

Pot No.	Soil type	Treatment	Dry weight of plants produced
			Gm.
356	Sand	Control	38.2
357	do	do	38.7
358	do	½ pound crimson clover	56.4
359	do	do	70.6
360	Hagerstown silt loam soil	Control	61.6
361	do	do	66.4
362	do	½ pound crimson clover	87.9
363	do	do	87.0

The results in Table 4 show that crimson clover has a marked effect on plant growth when turned under in the soil. Even with such inert material as sand the green manure increased plant growth, although the increase over a 3-year period was not so great as with the soil. With sand, there was very little effect the first year, but during the second and third years the effect was very marked. These results were obtained by using one of the best green-manure crops studied both in the greenhouse and in the field. The effect of rye on plant growth is probably not so good, as it is a nonlegume that gives various results, depending upon a number of factors, one of which is soil type, and therefore the following experiments were carried out on four of the leading Virginia soil types. These experiments were conducted in the same way as the preceding ones. (Table 5.)

The results of the experiments shown in Table 5 indicate that rye produced no marked effect in increasing plant growth. On the heavy clay soils of Albemarle and Appomattox there was a depression in plant growth. With the lighter soils of Norfolk and Blacksburg slight gains were observed from the turning under of rye. These experiments were planned as a check on a series of field experiments begun in 1912.

TABLE 5.—*Effect of green rye on different types of Virginia soil*

Plot No.	Soil type	Treatment	Dry weight (grams) 3-year average
298	Albemarle	Control	254.7
299	do	do	202.2
300	do	1 pound green rye	220.5
301	do	do	197.6
302	Appomattox Light	Control	226.8
303	do	do	242.7
304	do	1 pound green rye	205.7
305	do	do	182.3
306	Blacksburg	Control	250.0
307	do	do	233.5
308	do	1 pound green rye	286.7
309	do	do	348.5
310	Norfolk	Control	247.7
311	do	do	213.6
312	do	1 pound green rye	253.0
313	do	do	272.3

In the field work, 10 tenth-acre plots made up the series, 5 in corn and 5 in wheat. The green manures used were clover, rye, soy beans, and buckwheat. Results over a period of 12 years have been obtained with corn. The wheat was harvested on alternate years, and yields for six years are available. Tables 6 and 7 give the treatment, gain or loss in nitrogen, and the yields over the period 1912–1923:

TABLE 6.—*Yields of corn, in bushels per acre, for 12 years, under different methods of green manuring; and the effect of this treatment on the total nitrogen of the soil*

Plot No.	Treatment	Per cent nitrogen		Yield, 12-year average
		1912	1923	
1	Clover cut for hay	0.114	0.119	35.18
2	Clover turned under	.118	.125	37.48
3	Check	.076	.064	13.24
4	Rye cut for hay	.144	.090	17.25
5	Rye turned under	.112	.111	8.95

TABLE 7.—*Yields of wheat, in bushels per acre, for 6 years, under different methods of green manuring; and the effect of this treatment on the total nitrogen of the soil*

Plot No.	Treatment	Per cent nitrogen		Yield, 6-year average
		1912	1923	
1	Soy beans cut for hay	0.102	0.079	21.09
2	Soy beans turned under	.074	.123	26.02
3	Check	.074	.089	16.09
4	Buckwheat cut for hay	.090	.091	12.94
5	Buckwheat turned under	.098	.123	17.42

The data in Tables 6 and 7 show that with the possible exception of rye, the green manures have increased the yields of corn and wheat, and at the same time increased the total amount of nitrogen in the soil after giving up that necessary for the production of the crop.

Rye in the field and in greenhouse experiments has exerted a more or less harmful effect on plant growth, with corn as the indicator. This ill effect is apparently more or less temporary, and is probably due to the changes in the first stages of decomposition.

Clover showed a marked beneficial effect on the yield of corn and on the nitrogen reserve of the soil. The physical benefit derived from the turning under of this form of organic matter is very pronounced.

Rye did not increase the nitrogen reserve on plots 4 and 5, and when turned under it depressed the yield. The corn on the check and rye plots had a very yellow appearance, and gave every indication of nitrogen deficiency.

With wheat, green manures gave increases in all cases. This was very marked where soy beans were turned under. The soy-bean stubble did not increase the reserve nitrogen in the soil. The greatest increases in nitrogen were shown where soy beans and buckwheat were turned under, the soy beans giving 980 pounds of nitrogen per acre-foot and the buckwheat 500 pounds.

Lime was not included in the fertilization plan of these experiments, and a substantial addition of it to these plots is planned. It is believed that lime may counteract some of the injurious effects resulting from the incorporation of green rye with the soil. Recent laboratory results indicate that green rye does not nitrify as readily in the green state, but this same rye, when air-dried, gives increased initial nitrification when mixed with the soil. Several kinds of crops used as green manures exhibit this peculiarity. When green manures fresh from the field are mixed with the soil and kept at a definite temperature and with an optimum water content, an initial ammoniacal decomposition results. It may be that this rapid ammoniacal evolution causes the depressing effects often noticed when green manures are first turned under.

DECOMPOSITION OF GREEN MANURES IN THE SOIL, AS MEASURED BY CARBON DIOXIDE EVOLUTION

Analyses made over a period of three years of green manure crops cut and turned under at different periods of development have shown that such crops as rye, oats, clover, and vetch vary materially in composition and in their effects when incorporated with the soil.

At just what period of growth these crops should be turned under to furnish the most desirable form of organic matter is yet to be determined. An elaborate series of plots (80) has been under study at this station for some time, and the results so far obtained indicate that the younger and more succulent plants contain much more nitrogen and less crude fiber, or cellulose, than the more mature plants. The young, succulent plants decompose more rapidly in the soil than crops cut at a more mature stage.

All of the above points indicate that there is an intimate relationship between total carbon and the nitrogen of plants, and that the carbon and nitrogen exert a material effect on decomposition and consequently on the rate of carbon dioxide evolution. That the ratio of these two substances is to be taken into account has been indicated. Bearing these points in mind the following experiments were planned.

Rye, oats, clover, and vetch cut on May 17 when the plants were succulent, vigorous, and healthy, were selected as the forms of organic

matter to be studied. Cellulose in the form of filter paper was included in the series.

The plants were cut and taken to the laboratory, and the moisture was determined immediately. In every instance the equivalent of 0.5 gm. of the material was used, due allowance being made for the water content. The green materials were added to the equivalent of 100 gm. of dry Hagerstown loam soil, and water was added to bring the soils to optimum moisture content. The cellulose was added in like amount.

The soil and organic matter were placed in 500 c. c. Erlenmeyer flasks, and kept in the incubator at 25° C. The carbon dioxide was determined at daily intervals, due consideration being given to the proper flow and purification of the air passing over the mixture of soil and organic matter.

As a basis for future calculations of the rate of carbon dioxide evolution, and the ratio existing between the nitrogen and carbon of the organic matter, the amounts of total nitrogen and total carbon were determined. The methods of the Association of Official Agricultural Chemists were used (*1*). These data are shown in Table 8.

TABLE 8.—*Nitrogen and carbon in organic matter*

Substance, and date of cutting	Per cent nitrogen	Per cent carbon	Nitrogen-carbon ratio
Cellulose		44.44	
Rye, May 17, 1924	1.31	38.29	1 to 29.23
Oats, May 17, 1924	1.27	38.29	1 to 30.15
Clover, May 17, 1924	3.05	38.83	1 to 12.73
Vetch, May 17, 1924	3.98	38.92	1 to 9.78

The results given in Table 8 show that the nonlegumes contain much less nitrogen than the legumes, although the materials contained what might be regarded as the maximum amount of nitrogen for each plant at this period of its development. Rye cut at maturity (about June 15 for this locality) contains less than 1 per cent of nitrogen as has been indicated by previous work on the composition of this plant. The same is true of oats. There is a much wider ratio between the nitrogen and carbon of the nonlegumes than of the legumes; therefore one would expect a more rapid decomposition of the latter form of organic matter when mixed with the soil. Table 9 contains data bearing on this point.

In Table 9 no results are given for the soil blanks. These checks ran between 50 and 70 mg. of carbon dioxide for the 10-day period, and in every case the proper deduction was made, therefore the figures given above represent the entire amount of carbon dioxide evolved from the organic matter added in the form of green manure.

The cellulose-treated soil, where no nitrogen was present except that already present in the soil showed the lowest rate of decomposition, only 42.6 mg. of carbon dioxide having been liberated over a 10-day period. For the first three days of the experiment there was practically no decomposition going on. The maximum liberation of carbon dioxide was reached on the fourth day. Decomposition after that day was more or less uniform for the remainder of the experimental period.

TABLE 9.—*Decomposition of fresh green materials*

DAILY EVOLUTION OF CARBON DIOXIDE (MILLIGRAMS)

Substance	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
Cellulose	0.8	1.1	1.0	6.7	6.0	5.9	6.0	5.3	5.0	4.8	42.6
Rye	29.9	29.3	34.7	57.2	46.8	24.9	22.1	20.0	18.3	17.8	301.0
Oats	30.5	28.4	42.7	45.5	30.6	28.3	25.1	24.0	23.2	20.7	299.0
Clover	41.7	25.9	34.8	43.9	32.5	32.9	30.0	25.1	22.3	21.6	310.7
Vetch	46.4	49.5	65.4	72.0	35.1	39.6	27.4	26.1	25.0	22.1	408.6

DAILY DECOMPOSITION (PER CENT)

Cellulose	0.98	0.23	0.36	1.18	1.91	2.64	3.37	4.02	4.64	5.22	.....
Rye	4.26	8.43	13.37	21.51	28.17	31.72	34.86	37.71	40.31	42.84	.....
Oats	4.34	8.38	14.46	20.94	25.30	29.32	32.90	36.32	39.62	42.56	.....
Clover	5.85	9.49	14.37	20.53	25.09	29.71	33.92	37.45	40.58	43.60	.....
Vetch	6.50	13.43	22.59	32.68	37.59	43.14	46.97	50.63	54.13	57.23	.....

Rye and oats exhibited about the same rate of decomposition. The total for the rye was 301 mg. and for the oats 299 mg. However, there was a slightly greater liberation of carbon dioxide from rye than from oats, as the rye contained slightly more initial nitrogen than the oats. Rye and oats showed a much wider nitrogen-carbon ratio than clover and vetch. Rye and oats reached their maximum decomposition stage on the fourth day, with 57.2 and 45.5 mg. of carbon dioxide, respectively.

Clover and vetch showed greater carbon-dioxide evolution than rye and oats. Vetch gave off much more carbon dioxide than did clover. The maximum decomposition of both clover and vetch was reached on the fourth day. The initial nitrogen in the vetch was 0.93 per cent greater than that in the clover.

With the exception of cellulose, the decomposition of the several forms of organic matter was fairly rapid. The materials used were ground very fine, and all other conditions were favorable to rapid decomposition, probably much more so than would be experienced in the field; but there is every reason to believe that there would be almost as much if not more decomposition under field conditions, as the organic matter would be subjected to a much greater aeration and also to other atmospheric effects which can not be duplicated under artificial conditions. It would therefore appear that these experiments show carbon dioxide liberation closely approaching ordinary field conditions. Observations on clover added to soil in lysimeter rims have shown this material to be entirely converted into humus after a duration of about eight months.

Taking these results as a whole, the different forms of organic matter cut about May 17 give a fairly equal degree of decomposition, with the exception of vetch. Rye, oats, and clover released more than 40 per cent of their carbon dioxide during a 10-day period; with vetch, 57.23 per cent of the total carbon dioxide was evolved.

After the carbon dioxide had been determined in the samples, the mixtures were examined to determine the nature of the fermentation. All of the mixtures, with the exception of the untreated soil, gave a very pungent putrefactive odor, and there were evidences

of a strong ammoniacal fermentation. There was no germination of weed seed in any of the flasks, except the check to which no organic matter had been added, and in this flask the growth was exceptionally abundant.

Nitrate nitrogen determinations were made of the mixtures. The soil gave 2 mgs. of nitrogen as nitrate, and in all of the flasks to which organic matter had been applied not the slightest trace of nitrate nitrogen was found.

EFFECT OF AIR DRYING ON THE DECOMPOSITION OF GREEN MANURES

When the green manures were prepared for the preceding experiments, a certain portion was allowed to dry in air for subsequent study.

It has been thought for some time that there was a difference in the rate of carbon dioxide liberation in green organic matter as compared to the liberation from the same material after it had been thoroughly air dried.

In order to see just what effect air drying has upon green organic matter, a series of flasks was prepared duplicating the former experiments, using air-dried samples of rye, oats, clover, and vetch. The cellulose results of the former experiments were included for comparison. In all cases blanks with soil alone have been deducted from the daily evolution of carbon dioxide. The results are given in Table 10.

TABLE 10.—*Decomposition of materials after air-drying*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Substance	First day	Second day	third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
Cellulose	0.8	1.1	1.0	6.7	6.0	5.9	6.0	5.3	5.0	4.8	42.6
Rye	34.2	46.2	20.2	30.3	17.5	9.3	11.6	4.3	8.0	6.1	187.7
Oats	33.2	65.1	28.3	32.8	25.6	11.5	18.0	7.9	11.0	8.7	242.1
Clover	47.2	51.6	50.4	40.7	30.8	21.1	18.3	12.6	10.1	8.2	291.0
Vetch	39.9	63.9	43.0	64.4	35.9	17.0	20.8	12.3	8.1	7.0	312.3

DAILY DECOMPOSITION (PER CENT)

Cellulose	0.98	0.23	0.36	1.18	1.91	2.64	3.37	4.02	4.64	5.22	.....
Rye	4.87	11.44	14.32	18.63	21.12	22.45	24.10	24.71	25.85	26.72	.....
Oats	4.73	13.99	18.02	22.69	26.33	27.97	30.53	31.66	33.22	34.46	.....
Clover	6.62	13.87	20.94	26.65	30.98	33.94	36.51	38.27	39.69	40.84	.....
Vetch	5.59	14.54	20.56	27.06	32.09	34.47	37.39	39.10	40.24	41.22	.....

The results in Table 10 show that in every case air drying restricted the liberation of carbon dixoide as compared with the liberation from the materials fresh from the field. The loss in milligrams for the different treatments over a 10-day period, as compared with the green additions, was as follows: Rye 113.3; oats 56.9; clover 19.7; and vetch 96.3. Each form of organic matter, except vetch, reached its maximum stage of decomposition on the second day of the experiment. Air drying appeared to affect all treatments materially. This was especially noticeable with the legumes clover and vetch.

The fermentation of the mixtures was of a very different nature from that observed in mixtures of the green organic matter. There was no foul putrefactive odor, and all of the soil treatments were sweet and mellow. In every flask there was an abundant germination of weed seed. This was first observed on the fourth day, and the plants appeared to thrive as the experiment continued.

A chemical examination for nitrates showed their presence in every case, and a quantitative determination gave the following results in milligrams per 100 grams of dry soil: Soil 2; rye 0.3; oats 0.3; clover 1.35; vetch 1. There was a reduction of nitrates when compared with the soil which had received no organic matter. This loss of nitrates was in all probability due to the organism of the soils making use of it as food and as plant food supporting the young plantlets present in the soil.

In accounting for the difference in carbon dioxide liberation between the green and air-dried organic matter, it is thought that certain sugars may have been affected. There is strong probability that the more soluble carbohydrates which would serve directly as nutrients for the biological life necessary to proper decomposition had been changed. The changes in these bodies may have restricted the evolution of carbon dioxide.

In drying the plants it is also possible that the soluble hemicelluloses and other polysaccharides were changed into less soluble forms leading to the development of cellulose or wood fiber.

In an effort to substantiate the above hypotheses, it is planned to study these changes in subsequent experiments, by comparing the analyses of organic matter commonly used as green manures. The green, air-dried, and oven-dried substances will be studied to see if there are changes in the nitrogen-free extract and crude fiber. The different forms of green manure will be extracted with water and alcohol, and the extracts hydrolized with dilute hydrochloric acid, and the resulting invert sugars determined. It is believed that such a method of attack may throw some light on the changes brought about as the result of enzymic action or by the process of drying plant substances.

This work may show why a diminution in the liberation of carbon dioxide was obtained when air-dried forms of organic matter were allowed to decompose in the soil. In addition to these points, some light may be thrown on the subject of green manures being regarded by some as less efficient when turned under in an overripe condition.

EFFECT OF DATE OF CUTTING GREEN MANURES ON THEIR DECOMPOSITION IN THE SOIL

In the field work on green manures at this station, five cutting dates have been selected—April 15, May 1, May 15, June 1, June 15. Samples are not always obtainable on these exact dates, on account of the season, but cuttings made at times approximating these periods have been obtained since the beginning of the experiments. Table 11 shows the stage of development at which the crops were cut.

TABLE 11.—*Stage of development of green-manure crops*

Crop	Date of cutting	Stage of development
Winter rye	Apr. 15	6 to 8 inches high; beginning to joint.
Do	May 1	12 inches high and well jointed.
Do	May 15	Beginning to head.
Do	June 1	Milk stage.
Do	June 15	Nearly mature.
Winter oats	Apr. 15	4 to 6 inches high; beginning to joint.
Do	May 1	8 to 10 inches high, and well jointed.
Do	May 15	Beginning to head.
Do	June 1	Milk stage.
Do	June 15	Mature.
Crimson clover	Apr. 15	4 to 6 inches high.
Do	May 1	12 inches high.
Do	May 15	Clover in bloom.
Do	June 1	Blossoms beginning to die.
Do	June 15	Clover dead ripe.
Winter vetch	Apr. 15	8 to 10 inches high.
Do	May 1	15 to 18 inches high.
Do	May 15	Beginning to bloom.
Do	June 1	Vetch in full bloom.
Do	June 15	Setting seed.

The stage of development of the crops varied with the season, but the notes given in Table 11 will hold good on an average.

By means of crop yields and other studies, an effort is being made to determine the exact stage of development at which green-manure crops should be turned under to give the best results. For this work cuttings from rye, oats, clover, and vetch have been selected in order that their rate of decomposition might be determined.

Table 12 shows the percentages of nitrogen and carbon in the several forms of organic matter used in the carbon dioxide evolution experiments.

TABLE 12.—*Percentages of nitrogen and carbon in the green-manure crops, and the calculated nitrogen-carbon ratios*

Date of cutting of crop	Nitrogen	Carbon	Nitrogen-carbon ratio
Winter rye:	*Per cent*	*Per cent*	
May 14	1.21	34.95	1 to 28.88
May 21	1.03	35.23	1 to 34.20
May 28	.66	37.94	1 to 57.48
June 4	.81	39.27	1 to 48.48
June 18	.67	38.59	1 to 57.60
Winter oats:			
May 14	1.77	37.21	1 to 21.02
May 21	2.04	38.77	1 to 19.00
May 28	1.44	39.24	1 to 27.25
June 4	1.46	39.26	1 to 26.89
June 18	1.31	38.90	1 to 29.69
Crimson clover:			
May 14	3.09	36.80	1 to 11.91
May 21	2.51	43.13	1 to 17.18
May 28	1.79	38.33	1 to 21.41
June 4	2.12	36.52	1 to 17.23
June 18	1.98	38.84	1 to 19.62
Winter vetch:			
May 14	4.36	36.37	1 to 8.34
May 21	4.46	34.54	1 to 7.74
May 28	3.70	37.60	1 to 10.16
June 4	2.72	38.89	1 to 14.30
June 18	3.72	39.01	1 to 10.49

Table 12 shows that in general the younger cuttings of the crops used as green manures contained the greater percentages of nitrogen. Rye dropped materially in nitrogen composition after May 21, and

showed less than 1 per cent after that time. There was a gradual widening of the nitrogen-carbon ratios, and one would expect the rate of decomposition in the soil to be retarded as the crop approaches maturity. With oats, the first two cuttings gave the higher yields of total nitrogen, but after May 21 the nitrogen was fairly uniform. At maturity the oat crop contained less nitrogen than at any other stage. The legumes clover and vetch gave higher yields of nitrogen on the first two cutting dates. Total nitrogen throughout the five periods was much higher with vetch than clover, and consequently the nitrogen-carbon ratio was much narrower. With such a condition present, the vetch would be expected to decompose much more rapidly in the soil than the clover and thus give off more carbon dioxide.

Table 13 shows that the greatest total decomposition of rye was on the first cutting date. Rye cut June 18 contained about half the relative amount of nitrogen as that cut May 14. While the amount of nitrogen contained in the green manure undoubtedly has a relation to the rapidity of decomposition, the rate of disappearance may not be entirely dependent on the percentage of nitrogen. For instance, the decomposition of the crop cut on May 28 which contained 0.66 per cent nitrogen gave the same amount of carbon dioxide as the cutting of May 21, which contained 1.03 per cent nitrogen. On the next cutting date the amount of nitrogen was found to be 0.81 per cent, with a corresponding rise of 16.1 milligrams of carbon dioxide for the period of the experiment.

Taking the results as a whole, it appears that the greatest rate of decomposition is on or about May 14, and this would probably be the most ideal time for the turning under of this crop in order to get best results.

Comparing rye with other forms of green manure, it appears to decompose more slowly in the soil and therefore shows a much lower rate of carbon-dioxide evolution. In 10 days the youngest cutting gave up 43.54 per cent of its total carbon dioxide, against 27.61 per cent on the cutting of June 18, when the crop was mature.

Nitrate-nitrogen determinations were made of the mixtures after the liberation of the carbon dioxide, and the soil check contained 2.2 mg. of nitrogen as nitrate per 100 gm. of soil. In all of the rye treatments there was a reduction of nitrates, as only traces were found. There was a pronounced germination of weed seed in all of the cultures, and no foul putrefactive fermentation was detected.

TABLE 13.—*Decomposition of rye cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14	43.8	43.3	50.9	40.4	28.0	21.1	26.2	9.1	8.6	7.9	279.3
May 21	42.9	37.9	24.2	38.6	23.1	26.6	15.0	11.0	6.2	5.3	230.8
May 28	37.7	45.6	21.3	23.0	27.4	17.6	11.1	22.5	15.2	9.4	230.8
June 4	57.4	33.9	28.1	22.8	15.8	30.0	15.0	20.6	14.3	8.2	246.9
June 18	29.9	12.4	21.4	27.7	27.2	19.0	26.7	13.2	11.0	7.0	195.5

DAILY DECOMPOSITION (PER CENT)

May 14	6.82	13.58	21.51	27.81	32.17	35.46	39.55	40.97	42.31	43.54	.....
May 21	6.64	12.50	16.24	22.21	25.79	29.90	32.22	33.92	34.88	35.70	.....
May 28	5.42	11.97	15.03	18.33	22.27	24.80	26.39	29.62	31.81	33.16	.....
June 4	7.97	12.67	16.57	19.74	21.93	26.09	28.29	31.15	33.13	34.27	.....
June 18	4.22	5.97	9.00	12.91	16.75	19.44	23.21	25.07	26.60	27.61	.....

Table 14 shows that the first three cuttings of oats gave the greatest rate of carbon dioxide liberation over a 10-day period. The amount of carbon dioxide evolved was greater than that from rye with a maximum liberation from the oats cut on May 28. Taking the liberation of carbon dioxide as an index to the rate of decomposition under conditions existing in the field, the third cutting of oats is the best for use as green manures.

The initial nitrogen for oats was greater than that of the rye and as a result there was greater evolution of carbon dioxide.

Soluble nitrogen determination of these samples showed the soil blank to contain 4.8 mg. of nitrate to each 100 gm. of dry soil. With the mixtures the nitrate was present in sufficient quantity to get definite results, although there was a reduction in quantity over the untreated soil. In both cases nonlegumes showed a reduction of the nitrates already present in the soil. Germination was positive, and there was no foul putrefactive odor during the decomposition as was the case when green materials fresh from the field were used.

TABLE 14.—*Decomposition of oats cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14	59.9	31.8	73.1	59.1	43.0	16.4	5.2	7.9	3.3	3.2	302.9
May 21	115.5	39.0	40.6	32.4	31.0	24.2	11.7	8.8	4.0	2.4	309.6
May 28	140.6	56.7	61.6	30.8	15.6	24.6	4.7	3.0	1.8	1.4	340.8
June 4	50.7	44.7	64.0	30.0	28.1	26.3	7.4	10.1	6.3	3.8	271.4
June 18	42.9	80.8	45.7	28.4	17.6	6.8	14.5	5.4	4.6	3.6	250.3

DAILY DECOMPOSITION (PER CENT)

May 14	8.80	20.81	31.54	40.22	46.53	48.94	49.71	50.87	51.35	51.82	.....
May 21	16.23	28.74	34.45	39.00	43.36	46.76	48.40	49.64	50.20	50.54	.....
May 28	19.53	27.40	35.96	40.24	42.40	45.82	46.47	46.89	47.14	47.33	.....
June 4	7.04	13.24	22.12	26.29	30.19	33.84	34.86	34.88	37.14	37.67	.....
June 18	6.01	17.32	23.73	27.70	30.17	31.12	33.15	33.91	34.55	35.06	.....

The results in Table 15 show a greater evolution of carbon dioxide from clover than from either of the nonlegumes. This was especially noticeable with the cuttings of May 14 and 21. At this stage of growth the clover contained its maximum nitrogen. The greatest evolution of carbon dioxide was in the earlier days of the experiment, and it appears from these results that the young grass is decidedly the best for use as a green manure crop.

Residual nitrates were in greater quantity than in the case of the nonlegumes. The soil blank contained 0.13 mg. of nitrate nitrogen for each 100 gm. of dry soil, and there was an increase in the treated pots throughout the entire period of the experiment, the maximum nitrification being obtained with clover cut on May 21.

Germination of native seed in the soil was abundant in all of the flasks, and the fermentation was not of a foul, putrefactive nature.

TABLE 15.—*Decomposition of crimson clover cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14	98.7	92.7	81.2	18.3	29.3	22.0	21.0	11.3	10.1	8.7	393.3
May 21	97.3	81.8	59.2	43.5	31.4	30.1	33.4	4.7	3.4	2.1	386.9
May 28	78.0	45.0	53.7	27.2	20.4	21.4	17.6	9.8	2.0	1.7	276.8
June 4	74.0	51.8	42.9	39.8	13.0	12.3	38.3	8.4	5.2	4.1	289.8
June 18	42.1	48.4	31.9	25.2	19.5	20.3	30.4	8.7	1.2	1.0	228.7

DAILY DECOMPOSITION (PER CENT)

May 14	14.61	28.33	40.36	43.06	47.40	50.66	53.77	55.44	56.94	58.22	.....
May 21	12.29	22.63	30.11	35.60	39.57	43.37	47.59	48.18	48.62	48.88	.....
May 28	11.09	17.48	25.12	28.98	31.88	34.93	37.43	38.82	39.10	39.35	.....
June 4	11.04	18.78	25.18	31.12	33.06	34.90	40.61	41.87	42.64	43.25	.....
June 18	5.91	12.70	17.18	20.72	23.45	26.30	30.57	31.79	31.96	32.10	.....

The beneficial effect of winter vetch as a green manure is clearly indicated by the results in Table 16. There was a much greater decomposition throughout the entire series of cuttings than with any of the other crops experimented with. Maximum evolution of carbon dioxide was observed in every cutting of green manure either on the first or second day of the experiment. By the tenth day the evolution of carbon dioxide had become more or less uniform over the entire series. If the decomposition is an indication of the efficiency of the green manure, the cuttings of May 14 and 21 would be the preferable ones for turning under as green manure.

Germination of native seed in the soil was more pronounced in the vetch series than with any of the other crops tried out, and nitrate production was decidedly the greatest. The soil untreated contained 3.4 mg. of nitrate in each 100 gm. of dry soil, and the nitrates produced from the treated soils ranged from 13.2 to 26 mg. It was found that the younger the grass the greater was the production of nitrate.

TABLE 16.—*Decomposition of winter vetch cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14	96.8	94.8	53.7	40.6	36.4	30.2	28.6	26.4	18.7	10.9	437.1
May 21	105.1	56.8	49.4	55.0	48.0	36.1	28.4	26.3	20.7	18.9	444.7
May 28	101.6	105.7	50.8	42.7	39.9	30.0	20.6	15.2	10.0	5.1	421.6
June 4	54.1	81.3	34.7	23.2	18.0	16.9	15.4	13.8	12.6	11.8	281.8
June 18	120.7	42.9	54.7	35.6	15.7	10.1	9.3	7.4	6.3	5.9	308.6

DAILY DECOMPOSITION (PER CENT)

May 14	14.50	28.70	36.75	42.83	48.28	52.81	57.90	61.04	63.85	65.48	.....
May 21	16.58	25.54	33.33	42.00	49.57	55.21	59.75	63.90	67.16	70.14	.....
May 28	14.72	30.04	73.41	43.59	49.37	53.72	56.71	58.90	60.36	61.10	.....
June 4	7.58	18.99	23.84	27.09	29.61	31.98	34.14	36.08	37.84	39.50	.....
June 18	16.86	22.85	30.49	35.46	37.65	39.06	40.36	41.40	42.28	43.10	.....

Table 17 gives the total amounts of nitrate nitrogen and carbon dioxide developed over a 10-day period from the several forms of organic matter. It will be recalled that in the experiments using

green manure fresh from the field not a trace of nitrogen as nitrate was found. It was also noted that the nitrate in the soil was used up. Table 17 shows that the nitrate of the soil blanks varied more or less with each of the blanks. The soil blank for the oats contained the greatest amount of nitrate and that of the clover the least.

TABLE 17.—*Parallel formation of nitrate nitrogen and carbon dioxide in soils treated with green manures*

IN MILLIGRAMS OF NITRATE NITROGEN PER 100 GRAMS OF SOIL

Date green-manure crop was cut	Rye	Oats	Clover	Vetch
Soil blank	2.2	4.8	0.13	3.4
May 14	Trace.	1.6	13.4	26.0
May 21	Trace.	.5	6.0	12.0
May 28	Trace.	.5	1.0	18.0
June 4	Trace.	.5	1.0	18.6
June 18	Trace.	.5	1.0	13.2

MILLIGRAMS OF CARBON DIOXIDE FROM 0.5 GRAM OF ORGANIC MATTER

(Ten-day totals, with soil blanks deducted)

Soil blank	53.6	76.3	77.9	80.3
May 14	279.3	352.9	393.3	437.1
May 21	230.8	359.9	386.9	444.7
May 28	230.8	340.8	276.8	421.6
June 4	246.9	271.4	289.8	281.8
June 18	195.5	250.3	228.7	308.6

The nitrate nitrogen originally present in the soil was reduced in every instance by the nonlegumes rye and oats. An opposite effect was noted with the legumes clover and vetch. The greatest amount of nitrification was shown by vetch.

In former experiments (*3*) on nitrification it has been shown that there is a disappearance of nitrate nitrogen when cellulose is mixed with the soil. Such a condition will exist for probably 28 to 32 weeks, the reduction varying slightly with the soil type used. After about 32 weeks the injurious effect of the cellulose apparently passes off and nitrification then progresses slowly. It seems that there are certain types of bacteria that make use of the original nitrate of the soil for their early growth and maintenance, but after the reduction period of the first few weeks a gain is always noted.

The destruction of nitrates after cellulose is added to the soil has been reported by many investigators. Some claim that it is due to the process of denitrification, although total nitrogen analyses do not indicate a loss of nitrogen. Probably the cellulose furnishes a suitable medium for the growth of the organisms that convert nitrates into protein. In this transformation there is not any loss in total nitrogen, and in an ordinary nitrifying soil these protein compounds soon change to nitrates.

The same reduction phenomenon has been noted with rye and oats in the early stages of decomposition, but the soil recuperates much more rapidly with these forms of organic matter than with the more or less inert cellulose. It would seem, then, that legumes incorporated in the soil as green manures stimulate the formation of nitrate at a much earlier stage of their decomposition than do the nonlegumes,

which, when first turned under, apparently suppress nitrification for the first two or three weeks.

In reviewing the carbon-dioxide results, it will be seen that the young grass gave the greatest evolution of carbon dioxide when incorporated with a definite amount of soil. The readiness of decomposition was in the following order: Vetch, clover, oats, and rye. Taking the results as a whole, it appears that the green manures cut between May 14 and May 28 would be preferable as sources of organic matter for maintaining a high state of productivity.

For the nonlegumes, the ideal time for turning under would be when the plants were beginning to head. With vetch the best results are obtained when the plants are just beginning to bloom. Maximum efficiency with clover is obtained when the plants are in bloom.

SUMMARY

Pure cellulose applied to soils in different amounts restricted plant growth. This restriction was proportional to the amount of cellulose added. The addition of potassium nitrate in quantities of about 0.01 per cent failed to offset this harmful effect of the cellulose. Varying the quantity of nitrate had very little effect in counteracting the ill effects of the cellulose, beyond an 8-ton application.

Plants when grown in a well-balanced nutrient solution were healthy and vigorous, but the addition of cellulose depressed growth. Conditions were improved slightly by renewing the nutrient medium frequently. The depression in plant growth was proportional to the amount of cellulose added.

Decomposing cellulose produced hydrogen sulphide as a reduction product of the sulphates of calcium and magnesium contained in the nutrient solution.

The root systems of the plants were altered materially by a cellulose treatment, the roots becoming very much discolored and altogether unhealthy in appearance.

Crimson clover when added to sand and to Hagerstown silt loam soil in tubs and allowed to remain out of doors gave increased plant growth.

With four leading Virginia soil types, in tubs out of doors, rye in a green state depressed plant growth in the heavy clay types of soil. In the more open types of soil, a slight increase in growth was noted.

Corn grown in the field where clover was turned under gave an increased yield over corn with the clover removed for hay.

Corn was depressed in yield where rye was turned under over a period of 12 years.

Wheat grown where soy beans were cut for hay gave a much smaller yield than wheat which had soy beans turned under as a green manure.

Wheat yields were slightly increased by the turning under of buckwheat. There was a depression of yield when the buckwheat was removed for hay.

Rye, oats, clover, and vetch show a varied ratio between their nitrogen and carbon when cut on May 17. The ratio between the nitrogen and carbon is much wider for the nonlegumes than for the legumes.

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The evolution of carbon dioxide from rye, oats, clover, and vetch, cut on May 17 and mixed with soil, was much greater than from the same materials which had been allowed to air-dry for the same period of time. The legumes showed a more rapid decomposition in every case than did the nonlegumes.

When green materials were allowed to decompose in the soil, no nitrates were detected after the experiment had been run for 10 days. With the same substances that had been allowed to air-dry, nitrates were present, but in much greater quantity under a legume than under a nonlegume treatment.

Drying green manures retards their decomposition when gauged by carbon dioxide liberation over a 10-day period. This retardation in decomposition may probably be due to the soluble hemicelluloses and other polysaccharides having been changed to less soluble forms.

Rye, oats, clover, and vetch, cut at different stages of development, show wide differences in their nitrogen-carbon ratio. The younger the cutting the narrower the ratio. The ratio between these two elements widens as the plant approaches maturity. Vetch gave a much narrower ratio than any of the other crops studied.

Carbon dioxide liberation from green manures cut at different stages of growth showed a rapidity of decomposition in the following order: Vetch, clover, oats, and rye. Green manure cut between May 14 and May 28 showed the highest rate of decomposition. With cellulose in a pure form the decomposition was very slow. There was a fairly good nitrification with legume treatments, but with nonlegumes the nitrates formed were in much smaller amount.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1920. official and tentative methods of analysis. as compiled by the committee on revision of methods. revised to nov. 1, 1919. 417 p., illus. Washington, D. C.

(2) Baessler.
1893. untersuchungen über verschiedene gründüngungspflanzen und die wirkung derselben auf den ertrag der nachfrüchte. Wchnschr. Pommer. Ökonom. Gesell. 23: 227–231, 242–244.

(3) Hill, H. H.
1915. the effect of green manuring on soil nitrates under greenhouse conditions. Va. Agr. Expt. Sta. Tech. Bul. 6: 121–153.

(4) Hutchinson, C. M., and Milligan, S.
1914. green manuring experiment, 1912–13. Agr. Research Inst., Pusa Bul. 40, 31 p., illus.

(5) Maynard, L. A.
1917. the decomposition of sweet clover (melilotus alba desr.) as a green manure under greenhouse conditions. N. Y. Cornell Agr. Expt. Sta. Bul. 394: 119–149, illus.

(6) Merkle, F. G.
1918. the decomposition of organic matter in soils. Jour. Amer. Soc. Agron. 10: 281–302, illus.

(7) Pfeiffer, T., Frank, L., Friedlaender, K., and Ehrenberg, P.
1909. der stickstoffhaushalt des ackerbodens. Mitt. Landw. Inst. Breslau 4: 715–851.

(8) ———
1912. stickstoffsammelnde bakterien, brache und raubbau. Aufl. 2, 100 p. Berlin.

(9) SEELHORST, C. VON
1910. VERSUCHE ÜBER DEN VERBLEIB DES GRÜNDÜNGUNGSSTICKSTOFFS AUF EINEM SANDBODEN. V. Mitt. Deut. Landw. Gesell. 25: 291–292, 309–311.
(10) STARKEY, R. L.
1924. SOME OBSERVATIONS ON THE DECOMPOSITION OF ORGANIC MATTER IN SOILS. Soil Sci. 17: 293–314, illus.
(11) WAKSMAN, S. A., and HEUKELEKIAN, O.
1924. MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: VIII. DECOMPOSITION OF CELLULOSE. Soil Sci. 17: 275–291, illus.
(12) WHITE, T. H.
1916. TESTS OF THE USE OF CRIMSON CLOVER AS A GREEN MANURE FOR TOMATOES. Md. Agr. Expt. Sta. Bul. 199: 100–106.
(13) WHITING, A. L., and SCHOONOVER, W. R.
1920. THE COMPARATIVE RATE OF DECOMPOSITION OF GREEN AND CURED CLOVER TOPS IN SOIL. Soil Sci. 9: 137–149.